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(10) Robert E. Shope, M.D.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The identification of Rift Valley fever virus from Egypt and bluetongue virus from Australia had worldwide impact. Eighty-four other viruses were identified, many new to science or new to the geographic site of isolation.

Techniques were developed to study arbovirus attachment to neural and non-neural cells, to demonstrate high salt HA with dengue virus, to show HA in mosquito tissues with WEE virus, and to determine pathogenicity and serologic reactions to bunyavirus RNA-reassortants.

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20. Abstract, continued

Broadly based surveys of arbovirus antibody in man were done with sera from Brazil, Guam, USA, Colombia, and the New Guinea region. SLE antibody was detected in western Connecticut animals.

Arboviruses were not implicated serologically in Parkinson's disease, amyotrophic lateral sclerosis, or multiple sclerosis. Lyme disease was associated in distribution with Ixodes ticks but the etiologic agent was not isolated.

The reference center distributed 566 ampoules of reference sera, viruses, and antigens during 1977; mosquito and vertebrate cell lines were also distributed.

Annual Report for 1977

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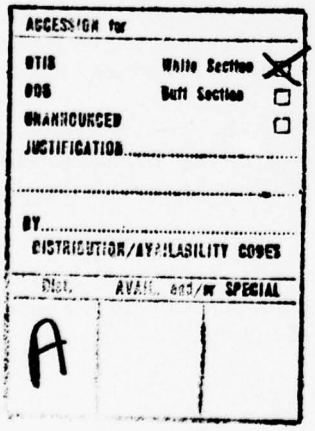

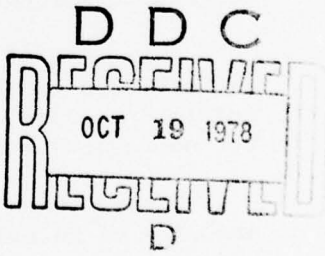

SUMMARY

The finding of Rift Valley fever virus in Egypt and Bluetongue virus in Australia have had great impact at opposite sides of the world. The Rift Valley fever epidemic is probably the largest, and certainly the most serious from the point of view of human death, ever recorded. Recognition of Bluetongue virus in Australia is of major significance to the sheep and cattle industries there. Other findings of note are:

Virus identification. Aroa virus from Venezuela and TR 127154 virus from bats in Trinidad are new group B agents. Tilligery is a new member of the Eubenangee group and Aus CH 16129 is new to the Simbu group. Both are from Australia. Other new viruses include the orbivirus, GG 668; a new bunyavirus from ticks, MI 19334; Termeil, Yacaaba, and PK 886 viruses from mosquitoes; and CSIRO-25 from Culicoides--all isolated in Australia. Another virus from ticks in Tanzania, RML 64423-8 appears to be new. Viruses which were isolated in a new geographic region were Thogoto from ticks, Sango from Culicoides, Dugbe from birds, Arumowot from rodents, and Germiston from sentinel mice, all from Ethiopia; Umbre and Thimiri for the first time in Australia; Arumowot from South Africa; and Tyuleniy from ticks of the North Atlantic. Recognition of eleven new rhabdoviruses led to a complete revision of this family by CF test. Two strains of SLE isolated by U.S. Army researchers in overwintering Culex were confirmed as to identity. In all, 86 viruses were identified in 1977.

Development of techniques and models. Techniques to study arbovirus attachment to neural and non-neural cells were developed as were methods to demonstrate high salt HA with dengue virus and HA in mosquito tissues with WEE virus. Animal models for determining pathogenicity and serologic reactions to Bunyavirus RNA-reassortants were established. Some reassortants had decreased pathogenicity for laboratory animals.

Serologic surveys. Broadly based surveys of arbovirus antibody in man were carried out with sera from Brazil, Guam, U.S.A., Colombia, West Irian, Papua, and New Guinea. Evidence was found for SLE antibody in western Connecticut animals.

Field Studies in Connecticut. Flanders and California group viruses were isolated from mosquitoes repeatedly. Isolation attempts from larvae yielded no evidence of transovarial transmission of Flanders virus.

Diagnosis of disease. Arboviruses could not be implicated serologically in Parkinson's disease, amyotrophic lateral sclerosis, or multiple sclerosis. Lyme disease was associated in distribution with Ixodes ticks but no agent was isolated from these ticks.

Distribution of reagents. The reference center distributed 566 ampoules of reference sera, viruses, and antigens during 1977; mosquito and vertebrate cell lines were also distributed.

FOREWARD

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

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I. Identification of Viruses from Human Outbreaks.

Rift Valley fever in Egypt. J. Meegan, J. Converse, O. Wood, J. Casals, and R. Shope. Five virus strains isolated from serum of patients in the early stage of an acute febrile illness were brought to YARU by Dr. Meegan on October 30, 1977. Dr. Imam Zaghloul Imam of the Egyptian Organization for Biological and Vaccine Production initially isolated agents from acute phase sera, throat washings, and blood-containing stool of patients at the Zagazig Hospital; Dr. Meegan at the NAMRU-III Laboratory in Cairo reisolated virus from five of these patients. According to fragmentary reports available at the time, an outbreak of the disease was currently occurring, since September 1977, in an area northeast of Cairo; the disease had affected thousands of persons, and as many as from 60 to 300 deaths with hemorrhage and jaundice had been attributed to the malady.

A rapid, presumptive identification of one of the strains, Eg-ZF 41, was accomplished by CF within 30 hours of arrival of the specimens at YARU. A stock suspension, mouse passage #2, brought from Cairo was used as antigen; this stock consisted of a 10% suspension of infected newborn mouse brain tissue in 0.75% bovine plasma albumin in buffered physiological saline. The stock--called undiluted-- was used in two-fold dilutions starting at 1:4. Dilutions, beginning at 1:4, of immune monotypic sera and of grouping polyvalent sera or ascitic fluids, were tested against the crude ZF-41 antigen and a similar control antigen. The test sera or ascitic fluids were: group A; group B; group Bunyamwera; group phlebotomus; polyvalent Bahig, Tete, Matruh, Matariya and Burg el Arab viruses; chikungunya; Rift Valley fever (RVF); Nairobi sheep disease; Germiston; and Eretmapodites 147. Only the RVF serum reacted positively with a titer of 1:16, against the suspension of ZF-41 diluted 1:4.

Newborn mice inoculated with the stock on arrival, sickened and were moribund 36 hours later. Antigens were prepared by the sucrose acetone method and by the freezing and thawing method from brain and liver tissues; an antigen prepared by acetone precipitation from the sera of the same mice agglutinated goose RBC at 1:1024, pH 5.75.

A CF test with the brain and liver antigens gave the result shown in Table 1; the tentative identification of ZF-41 as RVF was, therefore, confirmed.

An HI test done simultaneously using serum antigens for ZF-41 and Germiston viruses, gave the result illustrated in Table 2; not only did the HI test result coincide with that of the CF test, but in addition gave evidence of the presence of antibodies against ZF-41 in the serum of a patient.

In all, seventy-one human sera supplied by Dr. Imam through Dr. Meegan to YARU from patients of the Zagazig Fever Hospital were acetone extracted and HI tested for RVF antibody. Most sera were from the acute phase of the disease and were later found viremic. Four (#'s 5, 15, 16, 63) had HI antibody, the rest were negative at 1:10. Thirty of these were tested with group A and group B antigens in the HI test. Results were as follows:

Semliki	0/30
Sindbis	1/30
Chikungunya	0/30
Getah	0/30
Murray Valley	24/30
Central European TB	15/30
Dengue 2	16/30
Wesselsbron	15/30
West Nile	26/30

Highest titers were to West Nile antigen and were interpreted as representing prior exposure to this virus.

Fifty-five of the sera were also tested by CF with RVF brain sucrose-acetone antigen. Two (#'s 5, 15) were positive. Sera 16 and 63, positive by HI, were negative by CF.

Electron microscopy of thin-sectioned infected mouse liver and BHK-21 cells revealed typical Bunyavirus morphology (Figures 1-6).

Table 1. Complement-fixation Test: identification of strain ZF-41 as presumptive Rift Valley fever virus.

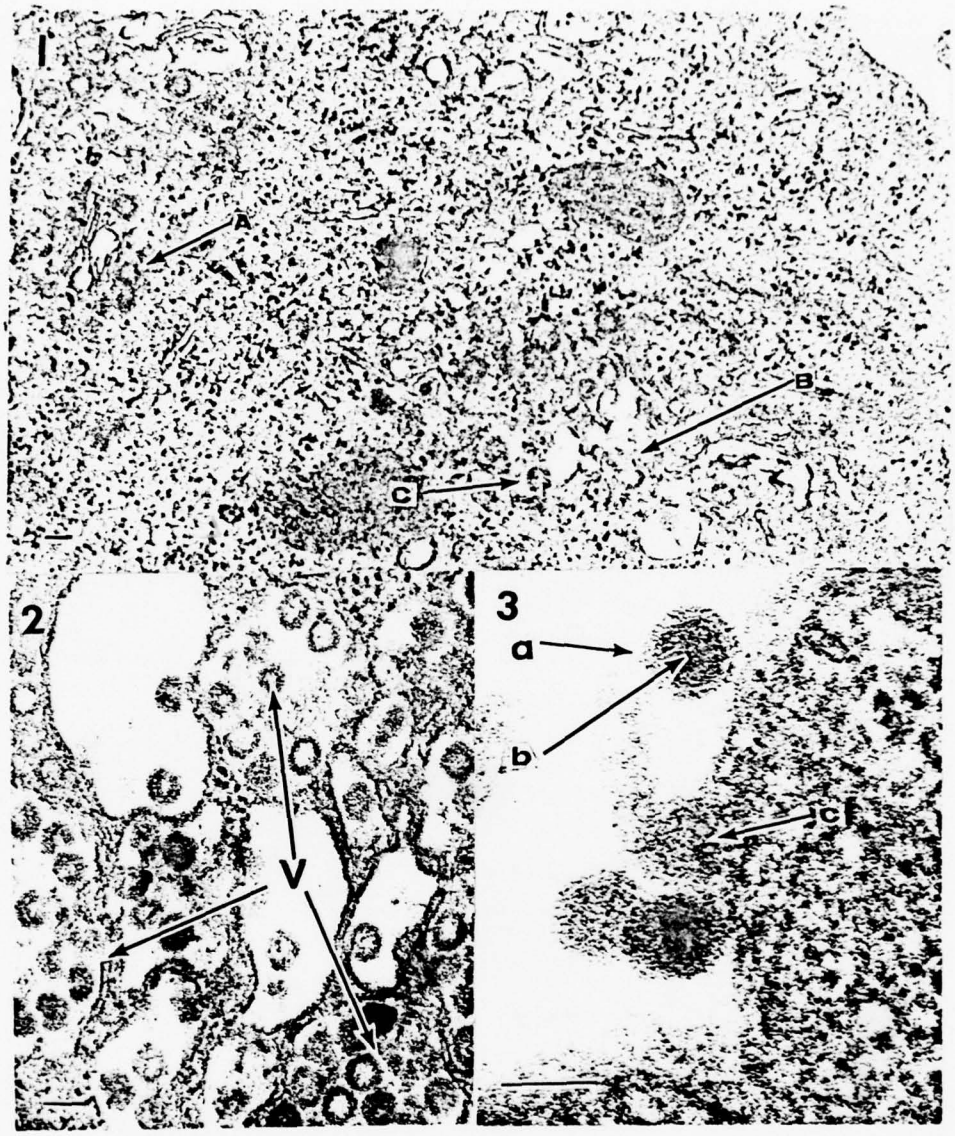
Antigen	Serum			
	RVF, sheep*	RVF, mouse	EMC	CHIK
ZF-41, Brain, sucrose-acetone	≥32/≥16	≥32/≥16	0	0
ZF-41, Liver, sucrose-acetone	≥32/≥32	≥32/≥32	0	0
ZF-41, Brain, frozen-thawed	≥32/ 8	≥32/≥16	0	0
ZF-41, Liver, frozen-thawed	≥32/ 16	≥32/ 64	0	0
Control, brain, sucrose-acetone	0	0	0	0
Control, Liver, sucrose-acetone	0	0	0	0

First dilution of serum and antigen, 1:4
*Supplied by U.S.Army, Fort Detrick, Maryland

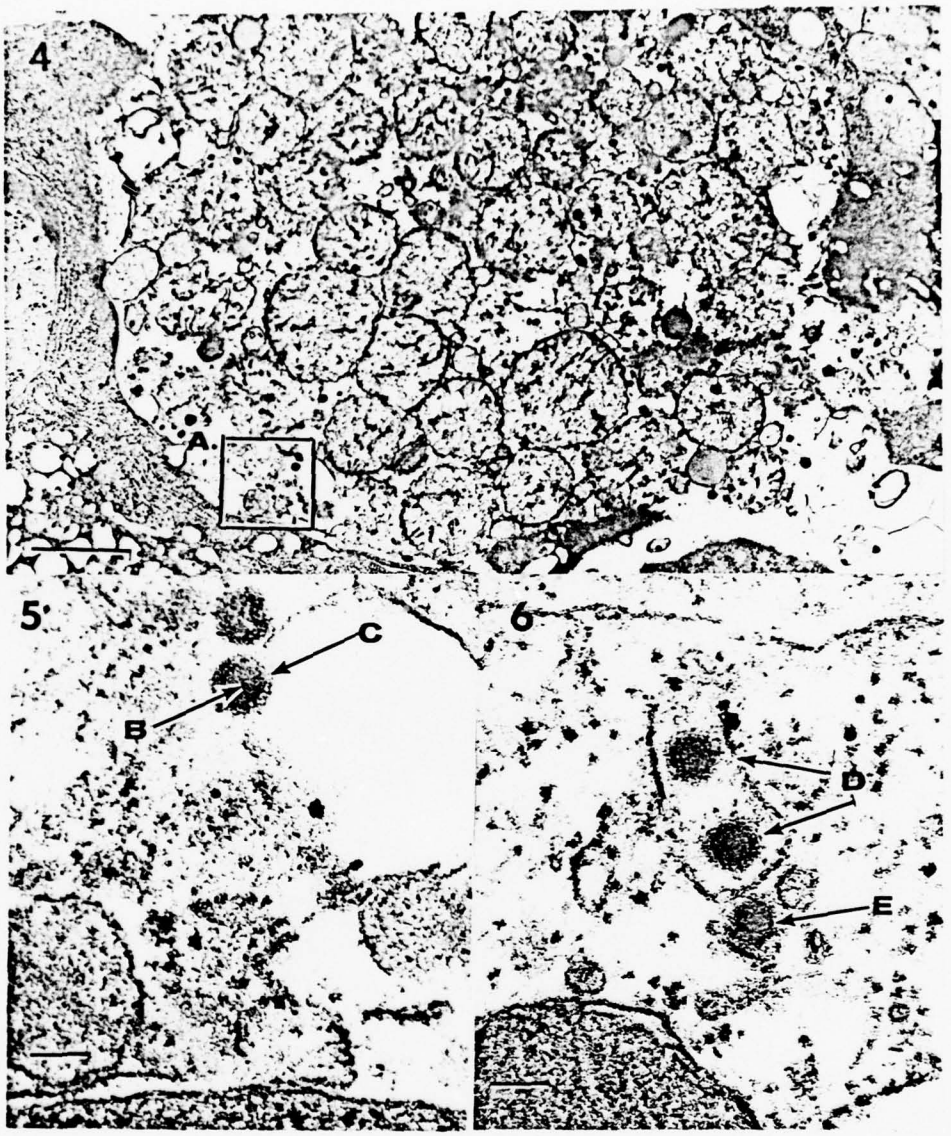
Table 2. Hemagglutination-inhibition Test: identification of strain Eg-ZF-41 as presumptive Rift Valley fever virus.

Sera*	Antigen	
	Eg-ZF-41 [4 units]	Germiston [2 units]
RVF, sheep	≥1:1280	0
RVF, mouse	1:160	0
Germiston	0	≥1:640
Zagazig fever, conv.man[#5]	1:640	0
Control, sheep	0	0
Zagazig fever, acute man[#'s 2,9-13]	0	0

First dilution of serum, 1:10
*Acetone extracted.



Figures 1-3 Rift Valley Fever Virus, Zagazig strain in BHK-21 cells 48 hours post infection. Figure 1 (40,000 X) shows a typical Bunyavirus replication complex. RVF virions are being formed in close association with vesicles of smooth endoplasmic reticulum at A and B. A virion appears to be budding into a vacuole at C. Figure 2 (47,000 X) shows cytoplasmic vacuoles (V) filled with large numbers of virions. Figure 3 shows the Bunyavirus morphology of RVF virions-- an envelope with fine spikes (a) and an apparently spherical core (b). A virion appears to be budding at c. Magnification in figure 3 is 142,500 X. The bar in the lower left of each figure is 100 nm.



Figures 4-6 Rift Valley Fever Virus , Zagazig strain in the liver of a suckling mouse moribund 39 hours after infection. Figure 1 (14,000 X) shows the remains of a cell destroyed in the infection. One can see the ballooned remnants of mitochondria and some virus particles. The box A is shown at greater magnification (85,500X) as Figure 5. The bar in the lower left of Fig. 4 is 1μm. Figure 5 (85,500X) shows particles with bunyavirus morphology a spherical core (B) and an envelope (C). Figure 6 shows two similar particles (D) in a vacuole near the edge of a cell. A third particle (E) appears to be attached to the vacuole membrane perhaps prior to budding into the vacuole. The bar in Figures 5 and 6 represents 100nm.

Dr. Shope visited Cairo December 13 and 14, 1977 as a WHO consultant. Information was obtained from Dr. Imam and other officials of the Ministry of Public Health and of the Animal Health Institute. The first human cases occurred in a six kilometer long irrigated zone of Sharqiya Governate bordered on the east by desert and extending north to Abu Hammad; later focal outbreaks in man occurred to the south in Qalyubiya Governate and to the west in Giza Governate, close to Cairo. October and November 1977 were hotter than normal. The epidemic zone supports citrus fruit and peanut cultivation. Culex pipiens made up almost the entire population of biting arthropods.

In retrospect, abortion in sheep and cattle was common starting in July, although this was not apparent on the sixth of October when human cases were first notified. It should be emphasized that Rift Valley fever has never before been recognized in Egypt and its clinical manifestations were entirely new to both the veterinarians and physicians. Hence, it was not considered in the differential diagnosis of the outbreak.

It is estimated that 70% of sheep and cattle aborted in the epidemic area during October. Isolations were made from sheep, cattle, buffalo, camels, and a horse. At least 20,000 persons were sick.

The typical human illness included fever, rigors, malaise, headache, muscle pains, early flushed face and eyes, conjunctival congestion, nausea and sometimes vomiting. Illness lasted from 2 to 5 days. There were 3 forms of complications: hemorrhagic fever and jaundice, often ending in death, ocular exudates and macular degeneration leading to blindness, and encephalitis with mortality in children. No information was obtained on human abortions.

The epidemic was believed to be arthropod-borne on epidemiologic grounds. Human cases apparently stopped in November after mosquito control measures were instituted, although animal disease was still being observed. Four members of a field team developed RVF four days after exposure to blood of a slaughtered sheep, indicating direct animal to man spread by aerosol.

RVF disease in animals may also be occurring in Upper Egypt, although laboratory confirmation is still lacking.

The basic questions of where RVF virus came from, what its epizootiology and epidemiology are, how widespread it is, and how to control it are still unanswered or only partly answered.

II. Identification of Virus Strains.

Viruses from Venezuela, Tanzania, Australia, Cambodia, and Ethiopia.

Aroa virus. I. Mattos, Robert Tauxe, and J. Casals. This agent appears to be a new group B virus; it was submitted by Dr. J. Castaneda, Director, Instituto de Investigaciones Veterinarias, Maracay, Venezuela,

under the designation, Ve NA 01809 (Aroa). The strain was isolated by Drs. Julieta de Siger and Norma Mettler, from a sentinel hamster exposed at Palma Sola, Yaracuy, Venezuela in 1972; the strain was sent to YARU in 1976 with the information that it was a group B arbovirus.

A sucrose-acetone antigen, active in the hemagglutination and complement-fixation (CF) tests, was prepared with the strain; also, an immune serum was obtained from mice given 2 immunizing injections and an immune ascitic fluid after 3 injections. The characterization of the virus has been done mainly by CF.

a. The group affiliation of Aroa virus was confirmed through the use of polyvalent immune reagents; only group B polyvalent fluid reacted with Aroa antigen, none of the other 6 fluids did.

b. A screening CF test was done in which Aroa immune serum (2 injections) in dilutions, beginning at 1:8, was tested against dilutions 1:4 and 1:16 of antigens for all the group B viruses, 52, in addition to the homologous antigen. The titer of Aroa serum against the homologous antigen was 1:64; with the other antigens the reactions were either negative or positive only at dilution 1:8, with the exception of antigens Alfuy, Bussuquara and Rocio (San Paulo encephalitis) with which reactions occurred with Aroa serum diluted 1:32 or, partial, 1:64.

c. A series of complete box-titrations, including antigen and immune serum for Aroa, Alfuy, Bussuquara and Rocio viruses indicated that Aroa differed substantially from the other agents, as shown in Table 3.

d. Additional CF results using immune ascitic fluids derived from mice given 3 vaccinating injections are shown in Table 4; and a complete box-titration comparing Aroa with Bussuquara, the group B virus with which it had consistently shown the most proximity, is shown in Table 5.

e. It is concluded that, by complement-fixation test, Aroa is a new virus of group B and that its closest relative in the group is Bussuquara.

US-RML-64423-8 (I. Mattos and J. Casals). The strain was submitted by Dr. Conrad E. Yunker, Rocky Mountain Laboratory, Montana, with the information that follows. The strain had been isolated from Argas brumpti ticks caught in Tanzania; its titer had not been affected by exposure to 5-bromodeoxyuridine; and it was not antigenically related to any of the viruses available at the RML.

An antigen and a mouse immune serum were easily prepared in the laboratory, the serum having a homologous titer of 1:64 or higher. Studies on the characterization of the virus have been carried out thus far by CF test. The serum in dilutions beginning at 1:4 or 1:8 has been screened against antigens in dilutions 1:4 and 1:16. The antigens used in the screening were for 311 of the 359 viruses listed in the "International Catalogue of Arboviruses, 1975"; missing in the tests were a number of viruses which may not be used in U.S. by regulations (for example, African Swine fever, African horsesickness, Marburg, Nairobi sheep disease, etc.) or a few that were not available at the moment in our laboratory. A partial list of antigens used is given in Table 6. All results were negative.

Table 3. Complement-fixation Test: characterization of Aroa Virus

Antigen	Serum			
	Aroa	Alfuy	Bussuquara	Rocio
Aroa	128/≥256	8/8	8/8	8/8
Alfuy	8/64	256/≥256	8/8	
Bussuquara	32/64	8/8	128/128	
Rocio	16/16			512/64

In this and subsequent tables the numerator expresses the reciprocal of the serum titer; the denominator the reciprocal of the antigen titer.

Table 4. Complement-fixation Test: characterization of Aroa Virus

Antigen	Serum
	Aroa
Aroa	128/≥32
St. Louis	32/≥32
Ilheus	16/≥32
Rocio	8/≥32
Bussuquara	64/≥32
Dengue 1	0
Dengue 2	16/8
US Bat	0
Control	0

Only dilutions 1:8 and 1:32 of antigen used.

Table 5. Complement-fixation Test: relationship between Aroa and Bussuquara

Antigen	Serum	
	Aroa	Bussuquara
Aroa	128/1024	32/512
Bussuquara	64/128	256/256
Control	0	0

0, no fixation at dilution 1:8 of serum and antigen.

Table 6. Antigens used in screening strains RML-64423-8 and Aus MI 19334.

Abu Mina	Colorado tick	Kowanyama	Silverwater
Acado	Corriparta	Kwatta	Simbu
Acara	Cotia	Lagos bat	Soldado
Akabane	Cowbone Ridge	La Joya	Sororoca
Amapari	Dengue 2	Lanjan	Sud An 754-61
Anhanga	Dera Ghazi Khan	Lebombo	Sud Ar 1169-64
Anopheles A	Dhori	Le Dantec	Tacaiuma
Anopheles B	Dugbe	Lone Star	Tamiami
Apeu	EEE	Lukuni	Tataguine
Arkonam	EgAn 1398-61	LCM	Tembe
Aruac	EgAn 1825-61	Manzanilla	Tensaw
Arumowat	EHD-NJ	Mapputta	Tete
Aus MRM 4059	Eretmapodites 147	Marco	Thimiri
Aus Ar 8659	Eubenangee	Matariya	Thogoto
Aus C 12048	Flanders	Matucare	Thottapalayam
Aus CH 9935	Gamboa	Melao	Timbo
Aus MK 6357	Germiston	Minititlan	Triniti
Aus MRM 10434	Gossas	Minnal	Trivittatus
Bahig	Grand Arbaud	Mirim	Tsuruse
Bakau	Guajara	MML	Turlock
Bandia	Guama	Modoc	Ug MP 359
Bangoran	Guaroa	Mokola	Umbre
Bangui	Hazara	Mossuril	Upolu
Bauline	Herpes	Mt Elgon bat	Utinga
BeAn 67949	Hilo	Nariva	Uukuniemi
BeAn 84381	Huacho	Navarro	Vaccinia
BeAn 100049	Hughes	NDV	VEE
BeAn 141106	I 6235	Nkolbisson	VS - New Jersey
BeAn 174214	I 61-2629	Nola	VS - Indiana
BeAn 177325	IbAn 8341	Nyamanini	Wad Medani
Bertioga	IbAn 20433	Nyando	Wanowrie
Bhanja	IbAn 28946	Okola	WEE
Bluetongue	IbAn 38918	Olifantsvlei	Witwatersrand
Bobia	Ichampadi	Oropouche	Wongal
Bocas	Ieri	Oyo	Wyeomyia
Boteke	Ilesha	Pacora	Yaba - 1
Botembe	Irituia	Pacui	Yata
Bujaru	Itaporanga	Palyam	Yellow fever
Bushbush	Itaqui	Pata	Yogue
Buttonwillow	Joinjakaka	Patois	Caraparu
Bwamba	Jos	Pichinde	Makindu
California	Junin	Powassan	Ug Sq 37317/9
Candiru	Jurona	Punta Toro	EgArT 904
Capim	Kaeng Khoi	Qalyub	Indo 1039
FG Ar 564	Kairi	Quaranfil	USSR Cq 13
Chaco	Kammavanpettai	Rabies	Ken T 83
Chagres	Kannamangalam	Reovirus	Tettnang
Chandipura	Karimabad	Rio Bravo	EgArT 1147
Changuinola	Kemerovo	Salehabad	Mal P 350
Charleville	Kern Canyon	SF - Naples	EgArT 427
Chenuda	Keterah	SF - Sicilian	Han 70-3-28
CHF-Congo	Klamath	Sathuperi	P 72-4R
CoAr 1279	Koongol	Sawgrass	BFN 3187
Cocal	Kotonkan	Sembalam	Oita 296

It is provisionally concluded that strain US-RML-64423-8 is an agent antigenically different and unrelated to any of the established arboviruses and to a few other viruses not transmitted by arthropods.

Aus-MI-19334 (I. Mattos and J. Casals). The strain was submitted by Dr. R. L. Doherty, Director, Queensland Institute of Medical Research, Brisbane, Australia; information accompanying the strain was as follows:

The strain had been isolated from Ixodes uriae, collected on Macquarie Island, Southern Ocean, on November 1975. The strain was easily maintained in newborn mice, passed through a 100nm Millipore filter but with marked loss of titer suggesting a size in the middle range (possibly a bunyavirus-like or orbivirus-like agent) and giving equivocal results on intrathoracic inoculation of A. aegypti with respect to replication in the insect.

Serological characterization of the virus in this laboratory has been carried out mainly by CF. A homologous antigen and serum system was easily prepared, with homologous titers of 1:256 for a serum, 1:128 to 1:256 for an ascitic fluid and 1:256 for the antigen.

a. Grouping ascitic fluids were tested in two-fold dilutions from 1:8 to 1:64 against dilutions of M1 19334 antigen from 1:4 to 1:32. The polyvalent fluids were for antigenic groups Kemerovo, Capim,Palyam, phlebotomus fever, Tete, California, VSV, Nyando, Guama, B, Tacaribe, Quaranfil and #2, 3, 4, 5, 6, 7, 10, and 12 (see Table 7). All reactions were negative.

b. Mouse immune serum or ascitic fluid for MI-19334, with homologous titers 1:128-1:256, were tested in dilutions beginning at 1:4 or 1:8, against a large number of arbovirus antigens (see preceeding section, strain US-RML-64423-8); the same antigens used for strain RML-64423-8 were used for MI-19334 (see also Table 6). The results of these tests was consistently negative; it is therefore concluded that M1-19334 is an agent distinct from and unrelated to any of the recognized arboviruses, presumably a new agent. By EM it had Bunyavirus morphology (p.47).

Cambodia M 127/69. (J. Casals). The strain was submitted by Dr. J. J. Salaun, sometime Director of the Pasteur Institute, Cambodia; it had been isolated in that country in 1969, from Aedes nocturnus, by mouse inoculation. According to information supplied by Dr. Salaun, a HA antigen prepared with the strain was not inhibited by polyvalent sera or ascitic fluids for groups A, B, and Bwamba, but was inhibited to a high titer by a Bunyamwera group reagent.

Antigen and antisera for the strain were prepared in this laboratory and following the lead supplied, the serum was tested by CF against representative viruses of Bunyamwera group and controls. The results of a test is given in Table 8, which indicates that there is a close relationship between this strain and Batai or Cache Valley. A complete box-titration CF test including M 127/69, Batai and Cache Valley, is shown in Table 9; evidently, M 127/69 is either Batai or Cache Valley by CF test. The closeness between the latter viruses has been well established by the

Table 7. Polyvalent grouping mouse ascitic fluids: viruses used in their preparation

#2.	Jurona, Minatitlan, MARU 11079, Gamboa, BeAn 141106
#3.	Koongol, Wongal, Bakau, Ketapang, Mapputta, Trubanaman, MK 7532
#4.	Nyamanini, Uukuniemi, Grand Arbaud, Thogoto
#5.	Hughes, Sawgrass, Matucare, Lonestar, Soldado
#6.	Marco, Timbo, Chaco, Pacui
#7.	Hart Park, Flanders, Kern Canyon, Klamath, Mt. Elgon bat
#10.	Upolu, DGK, Wanowrie, Dhori
#12.	Okola, Olifantsvlei, Witwatersrand, Dak Ar 1569, Tataguine

literature; in the present instance, and on the grounds of geographical considerations, it is tentatively concluded that Cambodia M 127/69 is a strain of Batai by CF test.

Eth Art 3559. (J. Casals). A strain submitted by Dr. Owen Wood, NAMRU-5, Ethiopia; isolated from Rhipicephalus evertsi collected in the lower Omo River Valley, Ethiopia. No CF system had been obtained due, presumably, to failure to obtain an antigen from infected mouse brain tissue.

A serum from mice repeatedly inoculated with the virus had been supplied by NAMRU-5; the serum in two-fold dilution from 1:8 to 1:64, was screened against antigens for 52 non-group B tick-borne viruses, used at dilutions 1:4 and 1:16. The only antigen with which serum Eth Art 3559 reacted was Thogoto, a liver antigen, with a titer of $\geq 64/>16$. In a subsequent test, the result of the screening was confirmed (see Table 10). No additional work was done here with the strain; its final characterization as a strain of Thogoto was done at NAMRU-5.

Eg Art 4137. (H.D.Watson and J. Casals). This strain was submitted by Dr. J. D. Converse, NAMRU-3, Egypt; it was isolated from Argas arboreus nymphs collected in Tanzania. The strain had been shown at NAMRU-3 to belong in the Hughes group and to be close to Punta Salinas.

Antigen and immune sera were prepared by standard methods and the strain was identified by CF tests.

a. Confirmation of group affiliation was easily obtained; an antigen for Eg Art 4137 was tested against seven grouping polyvalent ascitic fluids for groups B, Kemerovo, Quaranfil-Johnson and #4, 5, and #9 (see Table 7). Only fluid #5 reacted with the antigen; this fluid was prepared by immunizing mice with Hughes, Sawgrass, Matucare, Lonestar

Table 8. Complement-fixation test: identification of strain Cambodia M 127/69

	Serum
Antigen	M 127/69
M 127/69	32/128
Bunyamwera	0
Cache Valley	16/≥16
Batai	32/≥16
Ilesha	0
Germiston	0
Wyeomyia	0
SLE	0
Chikungunya	0

First dilution of serum 1:8, of antigen 1:4.

Table 9. Complement-fixation test: identification of strain Cambodia M 127/69

Antigen	Serum		
	M 127/69	Batai	Cache Valley
M 127/69	32/128	256/128	32/256
Batai	16/256	256/256	32/256
Cache Valley	16/128	128/256	64/256
SLE	0	0	0

Table 10. Complement-fixation test: identification of strain Eth Art 3559

Antigen	Serum	
	Eth Art 3559	Bandia
Thogoto, a	64/16	0
Thogoto, b	128/128	AC, 1/32
Thogoto, C	128/64	0
Caraparu	0	0
Marituba	0	0

and Soldado viruses. In a subsequent test, the antigen was tested with monotypic sera for the viruses in fluid #5, with the result shown in Table 11; this test showed that Eg Art 4137 is either Hughes virus or a member of the Hughes group.

b. Additional CF tests combined in Table 12, showed that Eg Art 4137 is indistinguishable from Punta Salinas and, although closely related to Hughes and Farallon, it is easily distinguishable from them.

Strains Ven-PAr 30318, Ven-PAr 30475, Ven-PAn 33382, Ven-PAn 33571, Ven-PAn 33574, Ven-PAn 33733, Ven-PAn 33706, and Ven-PAn 34958 (G. Calderon and J. Casals). These strains were submitted for identification by Dr. R. Walder, Instituto Venezuelano de Investigaciones Cientificas (IVIC); PAr 30318 and PAr 30475 had been isolated from mosquitoes, the remaining from sentinel hamsters by inoculation from insect suspensions or blood from hamsters to VERO cell cultures.

Immune sera or ascitic fluids were prepared for each strain, as well as sucrose-acetone antigens generally from brain tissue and in certain instances from liver tissue. The identification of some of the strains is still under way.

Placement in an antigenic group. As antigens became available, screenings were carried out with grouping polyvalent ascitic fluids; the combined results are given in Table 13. According to these results, strain PAn 33382 belongs in group C, strain PAn 33706 is as yet ungrouped and the five remaining isolates belong in group A.

Strain PAr 30318. An antiserum for this strain was screened against a number of group A antigens by CF test. The result of the screening showed that this isolate was indistinguishable from Una (Table 14), and is therefore provisionally considered to be a strain of Una virus.

Strain PAn 33382. An antiserum for this isolate was tested against a number of group C antigens, with the results shown in Table 15. From these results it would appear that isolate PAn 33382 could be a strain of either Caraparu or Itaqui; this conclusion was borne out by the result of a complete CF box-titration, Table 16. These two viruses are known to be nearly identical by CF test, but easily separable by the HI test. Unfortunately, HA antigens for PAn 33382 and Caraparu were not available at that time; since, however, it is also known that Caraparu and Apeu viruses are very closely related by HI, so antigen for the latter was used instead of an antigen for Caraparu in further efforts to identify PAn 33382. The result of these efforts is shown in Table 17.

Table 17 shows that the serum for PAn 33382 reacted with a titer of 1/640 with 8 units of Itaqui antigen and only with a titer of 1/40 with 2 units of Apeu antigen. If the titers for Apeu antigen given by the three sera in Table 17 were converted to their probable titers, had there been 8 units of this antigen instead of 2, the titers of serum PAn 33382 for antigens Itaqui and Apeu would have been 1/640 and 1/10 respectively; those of Caraparu serum, 0, i.e. less than 10, and 1:20; and the titers for Itaqui serum, similarly, 1/40 and 0.

Table 11. Complement-fixation test: identification of strain EgArt 4137

Antigen	Serum				
	Hughes	Soldado	Sawgrass	Matucare	Lonestar
EgArt 4137	≥64/≥64	0	0	0	0
Hughes	≥64/≥64	0	0	0	0

First dilution of serum and antigen, 1:8

Table 12. Complement-fixation test: identification of strain Eg Art 4137

Antigen	Serum				
	4137	P.S.	Farallon	Hughes	Zirqa
EgArt 4137	256/512	512/512	128/256	256/256	16/128
Punta Salinas	256/256	512/256			
Farallon	64/1024		512/1024		
Hughes	32/512	32/256	64/256	256/1024	8/128

Table 13. Complement-fixation test: identification of 8 strains from Venezuela

Antigen	Polyvalent serum or ascitic fluid						
	Group A	Group B	Group C	Group Buny	Group Cal	Group VSV	Homologous serum
PAr 30318	16/8	0	0	0	0		128/16
PAr 30475	≥64/≥32	0	0	0	0	0	
PAn 33571	≥64/≥32	0	0	0	0	0	
PAn 33574	≥64/≥32	0	0	0	0	0	
PAn 33733	≥64/≥32	0	0	0	0	0	
PAn 34958	≥64/≥32	0	0	0	0	0	
PAn 33382	0	0	≥64/≥32	0	0		≥64/≥32
PAn 33706	0	0	0	0	0	0	

Table 14. Complement-fixation test: identification of PAr 30318 as a strain of Una

Antigen	Serum or AF	
	PAr 30318, a	PAr 30318, b
PAr 30318	64/16	32/16
Mayaro	0	0
Una	256/≥8	64/≥8
Mucambo	0	0
Aura	0	0
VEE-Florida	0	0
EEE	0	0
WEE	0	0

First dilution of serum 1/8, of antigen 1/4

Table 15. Complement-fixation test: identification of strain PAn 33382

Antigen	Serum
	PAn 33382
Apeu	0
Caraparu	≥128/64
Gumbo Limbo	0
Itaqui	64/64
Madrid	0
Marituba	16/≥32
Murutucu	0
Oriboca	0
Ossa	0
Restan	0
Nepuyo	16/8, partial fixation
PAn 33382	128/≥64

First dilution of serum and antigen, 1/8

The CF and HI results are consistent with the probability that PAn 33382 is a strain of Itaqui, or of a virus very close to Itaqui; final identification of the isolate, however, awaits cross-testing PAn 33382 with both Itaqui and Caraparu by HI or neutralization tests.

Concerning the remaining strains, PAn 33571 and PAn 33574 have been found to be indistinguishable by CF test; and with these as well as with the others, work is being continued.

Table 16. Complement-fixation test: relationship between PAn 33382, Caraparu and Itaqui

Antigen	Serum		
	PAn 3382	Caraparu	Itaqui
PAn 33382	256/128	16/64	16/128
Caraparu	256/128	16/64	32/128
Itaqui	128/256	16/256	32/256
Gumbo Limbo	0	0	0

Table 17. Hemagglutination-inhibition test: identification of strain PAn 33382

Serum	Antigen	
	Itaqui, 8 units	Apeu, 2 units
PAn 33382	1/640	1/40
Caraparu	0	1/80
Itaqui	1/40	1/20

Investigation of possible serological differences among strains of St. Louis encephalitis virus from overwintering mosquitoes. I. Mattos and J. Casals. Two strains of presumed SLE virus were submitted by Dr. J. M Dalrymple, Walter Reed Army Institute of Research, Washington, D.C., with a request for confirmation of his tentative identification. Furthermore, if the strains turned out to be SLE virus, a serological comparison with other strains of this virus was suggested.

The strains submitted, isolated from Culex pipiens in midwinter were:

Ft. Mifflin: VP-34, SMB-3. (FM).
Ft. Washington: VP-7, SMB-2.(FW).

Antigens were prepared from infected newborn mouse brain tissue by the sucrose-acetone method; both strains readily gave hemagglutinating antigens with titers of the order of 1:1600 to 1:6400, and a pH range from 6.4 to 7.4, with an optimum between pH 6.8 and 7.2. These antigens were also active in the CF test with titers 1:256 or 1:512.

Immune sera were prepared in mice following two schedules of vaccination, as follows.

One-injection serum: mice IP inoculated with a dilution 10^{-2} of infected mouse brain tissue, 0.3 ml; bled out 20 to 26 days later. This type of serum was prepared for FM, FW strains; in addition similar sera are under preparation with SLE strains Parton, BeAr 23379 (a strain isolated in Brazil) and Bellis (a strain isolated in Argentina).

Two injection serum: mice IP inoculated with formalin inactivated virus on day 1, 0.3 ml 10^{-1}; on day 24-26, given the second injection of fully virulent virus, IP 10^{-1} 0.3 ml. Mice bled 8 days after the second injection and again, bled out, 32 days after the second injection. This type of serum has been prepared with FM and FW strains.

Confirmation of the preliminary identification was done by CF tests, the results of which are summarized in Tables 18 and 19.

In Table 18 is given the result of a screening of sera anti-FM and anti-FW, 1-injection, against antigens for several flaviviruses most likely to give marked crossing with SLE sera. The antigens were used in dilutions 1:8, 1:32 and 1:64; and the sera in two-fold dilutions beginning at 1:8.

The result of the test shows that on the basis of serum titers, both FM and FW strains are closer to SLE than to all other group B viruses used.

A complete box-titration was next carried out in which were included antigens and sera for FM, FW and SLE (Parton). The sera were all 1-injection sera; however, the Parton serum derived from guinea pigs instead of mice. The result of the test, Table 19, clearly shows that these three strains are indistinguishable among themselves; the lower titers of the antigens when tested with the Parton serum may be attributable to the fact that it was obtained from guinea pigs.

It appears therefore that, by CF, FM and FW are strains of SLE virus; it was decided to investigate whether they showed any antigenic difference when compared with a reference strain, Parton, in the kinetic HI test. The Parton strain was originally isolated in the U.S.

The timed - HI test was used as described for EEE virus in 1964 and applied since to several other togaviridae (RSSE, VEE, WN, SIN). The result of a test is shown in Table 20; the sera used were 1-injection sera.

Table 20 shows that antigen FW is not as sensitive as antigens for FM and Parton; this is shown by the fact that the FW serum consistently reacts with higher titers with either FM or Parton antigens than with the homologous one. Whether this lower sensitivity is a property of FW strain, or of this

particular antigen preparation, or associated with the difference in the numbers of transfers in the laboratory, cannot be stated without additional work. Insofar as FW strain is concerned, the antigen employed does not lend itself to the timed-HI test; therefore, no statement can be made with respect to similarity or difference between FW and the other two strains.

When Parton and FM strains are compared it is clearly apparent that at nearly every combination of serum dilution and antigen, particularly at 1/2 and 2 hours, Parton serum reacts better with its homologous antigen than with FM antigen; whereas FM serum reacts equally well with both FM and Parton antigens. On these grounds, FM and Parton strains are distinguishable from each other; the explanation of the difference between these strains is not obvious, perhaps FM has an antigenic determinant(s) that Parton does not have.

When antisera are available for strains BeAr 23379 and Bellis, it is planned to compare the five strains by plaque reduction test in VERO cells; and also, to compare FM and, if a better antigen is available, FW by kinetic-HI with the South American strains of SLE.

Table 18. Complement-fixation test. Identification of strains FM and FW

Antigen	Serum	
	FM	FW
Ft. Mifflin	128/≥64	128/≥64
Ft. Washington	128/≥64	128/≥64
SLE, Parton	64/64	64/≥32
JE, Nakayama	16/64	8/64
Ilheus, original	16/≥64	16/≥64
WN, EgAr 101	8/8	16/32
Bussuquara, An4073	8/8	8/8

Reciprocal of serum titer/reciprocal of antigen titer.

Table 19. Complement-fixation test. Identification of FM and FW as strains of SLE virus.

Antigen	Immune Serum		
	FM	FW	Parton
Ft. Mifflin	64/256	64/256	32/64
Ft. Washington	128/512	128/256	32/64
SLE, Parton	64/256	64/256	32/64

Reciprocal of serum titer/reciprocal of antigen titer.

Table 20. Kinetic HI test. Comparison of strains Parton, Ft. Mifflin and Ft.Washington.

Serum dilution		Reaction time in hours; antigen								
		1/2			2			18		
		P	FM	FW	P	FM	FW	P	FM	FW
Parton,	1/80	16	0	4	32	16	8	32	16	8
	160	0	0	0	16	0	0	16	8	4
	320	0	0	0	8	0	0	8	0	0
	640	0	0	0	0	0	0	0	0	0
Ft. Mifflin,	1/160	16	16	8	32	32	16	64	64	16
	320	0	0	4	16	16	8	32	32	8
	640	0	0	0	8	8	4	16	16	4
	1280	0	0	0	0	0	0	8	8	4
Ft. Washington,	1/160	0	0	4	32	16	8	64	32	16
	320	0	0	0	16	8	4	32	16	8
	640	0	0	0	0	0	0	16	8	4
	1280	0	0	0	0	0	0	0	0	0

Units of HA antigen inhibited.
0, less than 8 units for Parton and FM; less than 4 units for FW.

Viruses from Australia. G.Woodroofe,S.Buckley, R.Shope. Dr. Woodroofe of the Australia National University, Canberra identified 9 viruses from Australia during her stay as a visiting scientist at YARU, May and June, 1977.

Tilligery (NB 7080) virus was isolated from Anopheles annulipes at Nelson Bay and was shown to be an orbivirus by EM by Drs. Schnagel and Holmes (Aust. J. Biol. Sci. 28, 425, 1975), related by CF to Eubenangee, but different by NT according to studies of Dr. R. L. Doherty. A complement-fixation test at YARU confirmed the close CF relationship to Eubenangee and demonstrated a difference from Pata virus, the other member of the Eubenangee serogroup (Table 21).

Table 21. CF relationship of Tilligery virus to Eubenangee and Pata

Antigens	Ascitic fluids		
	Tilligery	Pata	Eubenangee
Tilligery	64/128*	8/8	0/0
Pata	8/2	256/128	0/0
Eubenangee	64/8	8/8	4/2
Normal	0/0	0/0	0/0

*Antibody/antigen

Termeil virus (BP8090) is one of 6 identical isolates from mosquitoes submitted as a virus unrelated to other Australian arboviruses. CF tests at YARU using a hyperimmune ascitic fluid to BP 8090 failed to show a relationship to over 200 antigens (see Table 6). A minor cross-reaction with Batai antigen was not confirmed in repeated tests.

Yacaaba virus (NB 6028) was isolated from Aedes vigilax, and in serological tests done in Australia was unrelated to 23 Australian viruses. CF tests at YARU with over 200 antigens (Table 6) were negative using a hyperimmune ascitic fluid prepared in Australia. Yacaaba virus is presumed to be a new ungrouped arbovirus.

BH_2 2193 virus from mosquitoes reacted by screening CF test with the Turlock grouping ascitic fluid. Definitive grid titrations indicated a very close CF relationship with Umbre virus of the Turlock group as shown in Table 22.

Table 22. CF reaction of BH_2 2193 virus with Umbre

Antigens	Ascitic fluids	
	Umbre	BH_2 2193
Umbre	16/128*	16/128
BH_2 2193	32/64	32/64
Normal	0/0	0/0

*Antibody/antigen

GG 668 and PK 886, both isolated from mosquitoes, were deoxycholate sensitive, had low titers i.c. in suckling mice but did not produce plaques in Vero cells or CPE in BHK 21 cells. Hyperimmune mouse ascitic fluids to GG 668 and to PK 886 were non-reactive by CF test with over 200 arbovirus antigens (Table 6). These viruses differ from each other and are presumed to be new ungrouped arboviruses. By EM GG668 is an orbivirus (page 46).

BW 9038 virus was isolated from mosquitoes. Screening CF tests revealed a cross-reaction with Wallal virus which was confirmed in reciprocal grid titrations. Titer of BW 9038 in baby mice was too low to carry out a satisfactory neutralization test. Attempts to adapt BW 9038 virus to BHK-21 cells were negative. This virus is presumed to be a strain of Wallal virus. BW9038 did not plaque in Vero cells.

WA 9302 was representative of 7 "isolates" from mosquito pools in Australia. In Australia, it was deoxycholate sensitive, HA negative and did not produce plaques in Vero cells. A reaction with the Bocas strain of mouse hepatitis virus was found in screening CF tests. Reciprocal grid CF tests with the SDA rat corona virus (indistinguishable by CF from mouse hepatitis virus) showed identity. It is presumed that WA 9302 is mouse hepatitis virus.

MD 9177 was an "isolate" from a mosquito pool. The antigen was screened with 23 grouping ascitic fluids and was positive with the fluid which contained ectromelia antibody. A grid CF titration confirmed that MD 9177 is a member of the vaccinia group, indistinguishable from ectromelia.

Bluetongue virus from Australia. C.L.Frazier with R. Shope. CSIRO-19 was isolated from a pool of Culicoides collected at Beatrice Hill and referred to YARU by Drs. H. Standfast, A. Dyce, T.St.George, and R. Doherty. In CF tests against the battery of grouping ascitic fluids the CSIRO-19 antigen reacted with two orbivirus fluids, polyvalent Palyam and polyvalent bluetongue. When the immune mouse sera to CSIRO-19 was tested against the antigens of the viruses in the two positive grouping fluids only the bluetongue antigen reacted positively. These results led to the testing of the CSIRO-19 antigen against a reference bluetongue (BT 8 strain) ascitic fluid from the WHO reference center and a bluetongue (BT 8 strain) reference ascitic fluid from the U.S.National Institutes of Health (Cat. No. U 519-701-562). The outcome of the test (Table 23) indicates that CSIRO-19 and Bluetongue are antigenically indistinguishable by CF test.

A high titered CSIRO-19 immune mouse ascitic fluid was reacted in CF with Orbivirus antigens from the WHO reference collection. Low level cross-reactions were detected with epizootic hemorrhagic disease of deer and Eubenangee viruses (Table 24).

This is the first time bluetongue virus has been recognized in Australia. Subsequent results from Onderstepoort, South Africa indicate that CSIRO-19 virus is a new bluetongue serotype, type 20. Recent studies in Australia have shown that the infection in cattle and buffalo is limited to the Northern Territory with no evidence to date of apparent illness in livestock. The finding of bluetongue virus in Australia has again validated the CSIRO Beatrice Hill surveillance project and has led to intensive study of bluetongue in Australia.

Table 23. Cross-reactions of CSIRO-19 and Bluetongue

Antigen	CSIRO-19	NIH Reference Bluetongue	WHO Reference Bluetongue
CSIRO-19	128/32*	128/32	32/32
Bluetongue	128/16	128/32	32/32

*Reciprocal of serum titer/reciprocal of antigen titer

Table 24. Reactions of CSIRO-19 antibody with Orbiviruses

Antigens	CSIRO-19 ascitic fluid
CSIRO-19	1024[a]
Epizootic hemorrhagic disease of deer	4
Eubenangee	8
Acado	0[b]
Palyam	0
Vellore	0
Kasba	0
Kemerovo	0
Tribec	0
Chenuda	0
Mono Lake	0
Haucho	0
Wad Medani	0
D'Aguilar	0
Orungo	0
Lebombo	0
Warrego	0
Mitchell River	0
Pata	0
Corriparta	0
Changuinola	0
Irituia	0
Colorado Tick Fever	0

[a]reciprocal of serum titer

[b]0=<4

Viruses from Australia, South Africa, and Thailand. C.L.Frazier with R. Shope. Unidentified isolates were passed in baby mice; a sucrose acetone extracted baby mouse brain antigen and an immune mouse ascitic fluid were prepared. Each antigen was tested with the grouping ascitic fluids listed in Table 25 by CF tests. The viruses from Australia were submitted by Dr. Ralph Doherty of the Queensland Institute for Medical Research.

CSIRO-25 was isolated from Culicoides peregrinus collected at Beatrice Hill in northern Australia. By CF test it reacted strongly with its homologous serum but failed to react with any of the grouping sera. CSIRO-25 remains ungrouped but is still under study.

CSIRO-1 was obtained from Culicoides histrio from Beatrice Hill while Ch 16129 was isolated from Culex annulirostris collected at Charleville. Both of the Australian viruses reacted with the Simbu grouping fluid in CF. The CF reactions of the two were examined with the members of the Simbu group (Table 26); Ch 16129 appeared to be a new virus distantly related to Mermet, Oropouche, and Utinga; whereas CSIRO-1 was closely related to Thimiri. Further CF testing with CSIRO-1 and Thimiri showed that the two were indistinguishable (Table 27). Neutralization tests performed in baby mice (Table 28) confirmed that CSIRO-1 and Thimiri were closely related or the same.

SA Ar 13532 was isolated from Culex rubinotus and referred to YARU by Dr. B. McIntosh of the South African Institute for Medical Research. Dr. McIntosh showed it to be related to Arumowot (Table 29). In the mouse neutralization test Arumowot virus was neutralized by Ar 13532 antibody but not vice versa (Table 30). This phenomenon could be explained by either of two hypotheses: first, that Ar 13532 and Arumowot are related in a one-way cross; second, that Ar 13532 is a mixture of two or more viruses, one of which is Arumowot.

BMK 1165-70 was isolated from Culex fuscocephala collected at San Sae, Chiang Mai Valley, Thailand. The virus was submitted by Walter Reed Army Institute of Research, for identification. The initial attempt to prepare a mouse brain antigen was not successful. However, a BMK 1165-70 immune mouse ascitic fluid reacted with antigens of Sango, Aino and Shuni viruses from the Simbu group. Further studies are in progress.

Tick-Viruses from Connecticut, Norway, Senegal, and South Africa. A. Main. Ar-159-77 and Ar-232-77 were recovered from ticks-Ixodes dammini and Dermacentor variabilis, respectively - in Connecticut during 1977, details on the collection and isolation of these strains are presented elsewhere in this report (Tables 63, 65). The two isolates were identical by CF but failed to fix complement with any of the antigens and/or specific or polyvalent ascitic fluids listed in Table 31. Subsequently, they were identified as mouse hepatitis virus.

Fin V-724, a group B isolate from Ixodes uriae in Norway, was submitted for identification by Dr. P. Saikku of the University of Helsinki. This isolate was identified by CF, HI, and NT tests as a strain of Tyuleniy virus (Table 32). This isolate extends the range of Tyuleniy to the Atlantic being previously reported from the Pacific (both Asia and North America) and the Arctic (Europe) Oceans.

Table 25

Grouping Ascitic Fluids Used in Attempts to Group Unknown Viruses

Group A
Group B
Group C
Group Bunyamwera
Group California
Group Kemerovo
Group Phlebotomus
Group Sakhalin
Group Tacaribe
Group VSV
Group Guama
Group Simbu
Group Capim

Polyvalent Groups
Anopheles A
Anopheles B
Turlock

Polyvalent Groups
Bwamba
Nyando
Mossuril

Polyvalent Groups
Koongol
Bakau
Mapputta

Polyvalent
Marco
Chaco
Timbu
Pacui

Polyvalent
Almpiwar
Belmont
Charleville
Japanaut
Joinjakaka
Kowanyama
Wallal
Warrego
Wongorr

Polyvalent
Johnston Atoll
Quaranfil
Kaisodi
Lanjan
Qalyub
Silverwater

Polyvalent
Rabies
LCM
Herpes
Vaccinia
NDV

Polyvalent
Congo
Hazara
Ganjam
Dugbe
Bhanja

Polyvalent
Patois
Zegla
Shark River
Mirim
Bertioga

Polyvalent
Bahig
Tete
Matruh
Matariya
Burg El Arab
EgAn 1398

Polyvalent
Nyamanini
Uukuniemi
Grand Arbaud
Thogoto

Polyvalent
Hughes
Sawgrass
Matucari
Lone Star
Soldado

Polyvalent
Hart Park
Flanders
Kern Canyon
Klamath
Mount Elgon Bat

Polyvalent
Navarro
Triniti
Aruac
Pacora

Polyvalent
Upolu
Dera Ghazi Khan
Wanowrie
Dhori

Polyvalent
Okola
Olifantsvlei
Witwatersrand
Bobia
Tataguine

Polyvalent
Jurona
Minatitlan
Gamboa
MARU 11079
BeAn 141104

Polyvalent
Palyam
Vellore
Kasba
Eubenangee
Corriparta
Acado
Pata
D'Aguilar

Polyvalent
Bluetongue
Epizootic Hemorrhagic
Disease of Deer
IbAr 22619
Changuinola
Irituia
Colorado Tick Fever

Table 26

CF Reactions of CSIRO-1 and Ch 16129 with viruses of the Simbu Group

Virus Antigens or Antibodies	Material reacted with Antigen or Antibody					
	Ascitic fluids		Sera		Antigens	
	CSIRO-1	Ch 16129	CSIRO-1	Ch 16129	CSIRO-1	Ch 16129
Mermet	8[a]	4	8	8	0	0
Oropouche	0[b]	8	0	8	0	8
Thimiri	4096	0	nd	nd	8192	0
Utinga	0	8	0	16	0	0
Manzanilla	0	0	0	0	0	0
Buttonwillow	0	0	0	0	0	0
Aino	0	0	0	0	0	0
Akabane	0	0	0	0	0	0
Sathuperi	0	0	0	0	0	0
Shuni	0	0	0	0	0	0
Sango	0	0	0	0	0	0
Sabo	0	0	0	0	0	0
Simbu	0	0	0	0	0	0
Nola	0	0	0	0	0	0
Yaba 7	0	0	0	0	0	0
Shamonda	0	0	0	0	0	0
CSIRO-1	4096	0	⩾128	0	--	--
Ch 16129	0	2048	0	⩾128	--	--
Simbu grouping fluid	--	--	--	--	32	32

[a] reciprocal of serum titer.

[b] <4

nd = not done.

Table 27. Cross-complement-fixation test between CSIRO-1 and Thimiri

Antigen	Ascitic fluid	
	CSIRO-1	Thimiri
CSIRO-1	4096/≥256*	8192/≥256
Thimiri	4096/≥256	8192/≥256

*reciprocal of serum titer/reciprocal of antigen titer.

Table 28. Neutralization test in mice: Identification of CSIRO-1 as Thimiri

Ascitic fluid	Virus			
	CSIRO-1		Thimiri	
	Titer	Log NI	Titer	Log NI
Normal	$10^{8.4}$		$10^{5.3}$	
CSIRO-1	$\leq 10^{1.5}$	≥6.9	$10^{2.4}$	2.9
Thimiri	$10^{3.1}$	5.3	$10^{2.7}$	2.6

Table 29. Cross-complement-fixation test between Ar 13532 and Arumowot

Antigen	Ascitic fluid	
	Ar 13532	Arumowot
Ar 13532	512/≥32*	8/256
Arumowot	512/≥32	8/256

*reciprocal of serum titer/reciprocal of antigen titer.

Table 30. Neutralization test in mice. Arumowot vs. Ar 13532

Ascitic fluid	Virus			
	Ar 13532		Arumowot	
	Titer	Log NI	Titer	Log NI
Normal	$\geq 10^{8.5}$		$\geq 10^{8.5}$	
Ar 13532	$10^{3.2}$	≥ 5.3	$10^{4.8}$	≥ 3.7
Arumowot	$\geq 10^{5.5}$	probably ≤ 3.0	$10^{4.9}$	≥ 3.6

Four strains of virus were recovered from Ornithodoros capensis group ticks collected at Langne-de-Barbarie, Senegal from a gull colony. All four isolates were identified by CF as strains of Soldado virus (Table 32).

SA Ar-15908 was submitted to YARU for identification by Dr. B. McIntosh of the South African National Institute for Virology. The strain was recovered from Ornithodoros ticks collected at Lamberts Bay, Cape Town and identified by CF as Soldado virus (Table 33). Soldado virus has now been reported from Trinidad (Caribbean), Wales (North Atlantic), Ethiopia (Lake Shalla), Senegal, and South Africa.

Table 31. Antigens and/or Ascitic Fluids used in CF tests with Ar-159-77 and Ar-232-77

Aruac
AusMK 5325

Bahig
Bakau
Bandia
Bhanja
Bluetongue
Burg el Arab
Bwamba

Cache Valley
Changuinola
Chobar Gorge
*Clethrionomys gapperi virus
Colorado Tick Fever
Congo-Crimean Hemorrhagic Fever
Cowbone Ridge

Dak 1569
Dera Ghazi Khan
Dhori
Dugbe

*Eastern Equine Encephalitis
EgAn-1398-61
Epizootic Hemorrhagic Disease of Deer

*Flanders

Ganjam
Grand Arbaud

Hart Park
Hazara
Herpes
Hughes

IbAr 22619
Irituia

*Jamestown Canyon

Kaisodi
Kern Canyon
Keterah
Keystone
Klamath
Koongol

Lone Star
Lymphocytic choriomeningitis

Mapputta
Matariya
Matruh
Modoc
Mossuril
Mount Elgon Bat

Navarro
Newcastle Disease
Nyamanini

Okola
Olifantsvlei

Pacora
Powassan

Quaranfil

Rabies

Sapphire II
Sawgrass
Silverwater
*Snowshoe Hare
Soldado
St. Louis Encephalitis

Tataguine
Tensaw
Thogoto
Triniti
Trivittatus
Trubanaman
Tyuleniy

Upolu
Uukuniemi

Vaccinia
Vesicular stomatitis-Indiana
Vesicular stomatitis-New Jersey

Wad Medani
Wanowrie
*Western Equine Encephalitis
Witwatersrand
Wongal

Group California
Group Hughes
Group Kemerovo
Group Sakhalin
Group Uukuniemi

* local antigens.

Table 32. CF, HI, and NT Tests comparing FinV=724, Tyuleniy (LEIV 6c), and Saumarez Reef (CSIRO-4) viruses.

Complement-fixation test:

Antigen	Antibody: FinV-724 (MAF)	Tyuleniy (serum A)	Tyuleniy (serum B)	CSIRO-4 (MAF)
FinV-724	128/128	64/256	32/128	32/32
Tyuleniy	128/128	64/32	32/32	16/4
CSIRO-4	128/32	64/32	16/16	128/256

Reciprocal of serum titer/reciprocal of antigen titer.

Hemagglutination-inhibition test:

Antigen	Antibody: FinV-724	Tyuleniy (serum A)	Tyuleniy (serum B)	CSIRO-4
FinV-724	2560	320	320	160
Tyuleniy	320	160	80	40
CSIRO-4	1280	640	640	640

Reciprocal of serum titer

Neutralization tests (suckling mouse):

Virus	Antibody: FinV-724		Tyuleniy (USSR-LEIV 6c)		Saumarez Reef (Aus CSIRO-4)		Normal
	LLD_{50}	LNI	LLD_{50}	LNI	LLD_{50}	LNI	LLD_{50}
FinV-724	2.4	4.6	3.4	3.6	5.4	1.6	7.0
USSR-LEIV 6c	3.1	4.7	3.1	4.7			7.8
Aus CSIRO-4	6.0	2.2			4.4	3.8	8.2

Table 33. Complement-fixation Tests: Hughes Serogroup

Antigens	Ascitic Fluids									
	SenAr-386-77	SAAr-15908	Soldado	Hughes	Farallon	Zirqa	Punta Salinas	Sapphire II	Group Hughes*	Normal
SenAr-386-77	256/64	16/16	512/64	0	0	0	0	0	16/64	0
SAAr-15908	128/128	32/32	128/32	0	0	0	0	0	8/16	0
Soldado	256/512	16/32	≥512/128	16/8	0	0	0	0	32/128	0
Hughes	0	0	0	≥512/256	0	8/64	64/256	0	128/512	0
Farallon	0	0	0	64/128	32/128	32/128	64/128	0	32/256	0
Zirqa	0	0	0	16/64	0	512/256	16/64	0	8/64	0
Punta Salinas	0	0	0	128/64	4/32	16/64	≥512/256	0	128/128	0
Sapphire II	0	0	0	0	0	0	0	512/512	0	0
Normal	0	0	0	0	0	0	0	0	0	0

Antigens	Ascitic Fluids				
	SenAr-386-77	SenAr-387-77	SenAr-388-77	Group Hughes*	Normal
SenAr-386-77	256/64	512/64	≥512/64	16/64	0
SenAr-387-77	128/128	≥512/128	≥512/128	16/64	0
SenAr-388-77	256/128	≥512/128	≥512/128	16/128	0
SenAr-389-77	256/128	512/64	512/64	-	0
Normal	0	0	0	0	0

*Mice inoculated with Hughes virus, Soldado virus, and Farallon virus.

El Delerio virus from Venezuela. S. Hildreth, G. Roze, and R. Shope. A strain of EEE virus from Venezuela was referred for subtyping by Dr. J. Castaneda of Maracay. CF and HI tests confirmed that El Delerio virus was more closely related to EEE than to other group A viruses. The CF test indicated that El Delerio hyperimmune ascitic fluid reacted to higher titer with an Argentina EEE strain than with the Ten Broeck strain (Table 34). An HI test confirmed the easy differentiating of El Delerio from North American EEE strains (Table 35). By N test, El Delerio virus was neutralized 3.8 log LD_{50} by EEE (Ten Broeck) ascitic fluid.

Table 34. CF test with the El Delerio strain of EEE virus

Antigens	Antibody El Delerio
El Delerio	256
EEE (Ten Broeck)	32
EEE (Argentina)	128
WEE	16
VEE	16

Table 35. HI test with the El Delerio strain of EEE virus

Antigens	El Delerio	EEE (Ten Broeck)	EEE (Massachusetts)	WEE	SLE
El Delerio	320	80	20	0	0
EEE (Ten Broeck)	80	1280	640	0	0

New group B virus from Trinidad Chilonycteris bats. A. Porter, G. Roze, R.Shope. TR 127154 virus was referred by Dr. John L. Price of the Trinidad Regional Virus Laboratory. It was isolated from pooled brains and salivary glands of 6 Chilonycteris rubiginosa captured in Tomana cave 12/09/73. Dr. Price observed low-titer CF reactions of TR 127154 antigen with NIH group B ascitic fluid, but could not confirm the relationship. CF tests at YARU with a battery of grouping reagents failed to group TR 127154 virus. A hemagglutinating antigen with optimal pH of 6.4, however, was inhibited by acetone extracted ascitic fluids of group B and West Nile (Table 36). Another test with antigens of group B viruses and TR 127154 hyperimmune ascitic fluid confirmed the group B relationship and indicated greater cross-reaction with antigen of Montana Myotis leukoencephalitis virus than with other agents in group B.

Table 36. HI relationship of TR 127154 virus with group B.

Ascitic fluid	Titer
yellow fever	<10
Cedros	<10
Dengue 2	<10
West Nile	20
TR 127154, Tap 4	≥160
phlebotomus group (NIH)	<10
Candiru	<10
TR 127154 pool	≥160
Rio Bravo	<10
Naples	<10
phlebotomus group (YARU)	<10
Group B	20
St. Louis	<10

Viruses from Ethiopia. O. Wood and D. Winograd. The Naval Medical Research Unit #5 submitted for identification, viruses isolated from arthropods and animals as part of a broad ecologic study of disease in Ethiopia. Most of the hyperimmune mouse sera and antigens were prepared in Ethiopia prior to the termination there of laboratory activities in April, 1977.

Antigens of eleven strains reacted with the group B grouping fluid by CF:

Eth An 1334	Bird
Eth An 3307	Bird
Eth An 3662	Bird
Eth An 4152	Bird
Eth An 4766	Bird
Eth An 4769	Bird
Eth An 4811	Bird
Eth An 4733	Bird
Eth An 4767	Bird
Eth An 4768	Bird
Eth Ar 4698	Mosquitoes

Sera of seven of these viruses were further reacted by CF with 19 antigens of group B viruses including those known to be in Africa. In each case the highest titer was to West Nile antigen with lesser reaction to Usutu, Japanese encephalitis, Zika and Israel turkey meningoencephalitis viruses. Table 37 shows reciprocal CF test results of grid titration with West Nile antigen and antibody. In addition, Eth An 4733, Eth An 4767, Eth An 4768, and Eth An 4698 reacted by CF to titer with West Nile serum.

In plaque reduction neutralization tests with the Egypt 101 strain of West Nile virus, sera of Eth An 3307, Eth An 3662, Eth An 4152, Eth An 4811, Eth An 4766, and Eth An 4769 gave 50% or greater reduction at >1:512. In addition the titers of Eth An 4733, Eth An 4767, and Eth An 4768 were reduced 2 or more logs by a West Nile reference serum, thus confirming the identification of these strains of West Nile virus.

Table 37. CF reactions between West Nile and viruses from Ethiopian birds

	WN	1334	3307	3662	4152	4766	4769	4811
WN	2048*	256	>512	512	256	512	64	64
1334	1024	128						
3307	1024		>512					
3662	1024			256				
4152	1024				256			
4766	1024					256		
4769	1024						64	
4811	1024							32
Normal	0	0	0	0	0	0	0	0

*Reciprocal of serum titer.

Seven strains of virus from sentinel mice exposed 12 through 30 November, 1976 in Bulaka Forest on the eastern shore of Lake Maguerita had similar incubation periods; antigen of one of these viruses, Eth An 4865, Eth An 4867, Eth An 4870, Eth An 4873, Eth An 4874, and Eth An 4876 were shown to react by CF (1:128) with Germiston ascitic fluid (homologous titer 1:128) and are presumed to be strains of Germiston virus.

Eth Ar 2316 was isolated from Culicoides. CF tests in Ethiopia with grouping fluids placed it in the Simbu group; another test done at YARU (Table 38) showed it to be closely related to Sango and Shuni viruses. Sango and Shuni viruses are separable by HI but not by CF. A sucrose-acetone extracted mouse brain antigen failed to hemagglutinate using standard 0.15 M NaCl-phosphate buffers; however, using 0.4 M NaCl-phosphate buffer a titer of 1:64 at pH 6.0 was obtained. HI test results (Table 39) identified Eth Ar 2316 as a strain of Sango virus, the first time this agent has been recognized from Ethiopia.

Table 38. CF reactions of Eth Ar 2316 serum with Simbu group antigens

Antigens	Eth Ar 2316 serum	Antigens	Eth Ar 2316 serum
Eth Ar 2316	256*	Simbu	64
Sabo	128	Sathuperi	64
Utinga	<8	Manzanilla	<8
Buttonwillow	<8	Sango	512
Oropouche	<8	Shamonda	64
Yaba 7	64	Shuni	256
Akabane	64	Thimiri	<8

*Reciprocal of serum titer.

Table 39. HI reactions of Eth Ar 2316 and Simbu group viruses

Antigens	Antibody							
	Eth Ar 2316	Sango	Shuni	Akabane	Sabo	Sathuperi	Shamonda	Simbu
Eth Ar 2316	80	40	<10	<10	<10	<10	<10	<10
Sango	80	80						

Eth An 798 and Eth An 808 were isolated from Arvicanthis niloticus (the Nile rat). Antigens of these two viruses reacted by CF test in Ethiopia with the phlebotomus fever grouping fluid. At YARU serum of Eth An 808 (homologous CF titer 1:32) reacted 1:32 with Arumowot, 1:16 with Naples and <1:8 with Karimabad, Sud An 754-61 and Sicilian antigens. Subsequent grid CF tests showed Eth An 798 and Eth An 808 to be strains of Arumowot virus (Table 40), or very closely related.

Table 40. CF tests of Eth An 798 and Eth An 808 with Arumowot virus

Antigen	Antibody		
	Eth An 798	Eth An 808	Arumowot
Eth An 798	32/256	64/>256	8/128
Eth An 808	32/64	64/>512	32/>512
Arumowot	32/512	256/>512	32/>512
Normal	0/0	0/0	0/0

Eth An 4731, Eth An 4732, and Eth An 4734, Eth An 4735, Eth An 4736, Eth An 4737, Eth An 4738, Eth An 4739, Eth An 4740, Eth An 4741, Eth An 4742, Eth An 4743 were isolated from organs of birds collected during 1975 at Koka, Ethiopia. These viruses were indistinguishable by CF test inter se. Antigen of Eth An 4731 was chosen as representative, and this antigen reacted with the grouping fluid containing Congo, Dugbe, Ganjam, Bhanja, and Hazara antibody. Testing with individual antigen or sera revealed a close CF relationship of Eth An 4731, Eth An 4732, Eth An 4734, Eth An 4735, Eth An 4736, Eth An 4737, Eth An 4738, Eth An 4739, Eth An 4740, Eth An 4741, Eth An 4742, and Eth An 4743 with Dugbe virus.

Serological Classification of rhabdoviruses. C. L. Frazier and R. Shope. Several newly recognized rhabdoviruses visualized by EM by Dr. F. Murphy, CDC, Dr. A. El Mekki, Institute for Tropical Medicine, Antwerp, Dr. N. Stanley, Perth, Australia, Dr. F. Pinheiro, Belem, Brazil and at YARU have been included in a study to compare all known rhabdoviruses of vertebrate animals by complement-fixation test. The study is not yet complete. Sucrose acetone extracted mouse brain antigens and 4-inoculation mouse ascitic fluids were compared in screening tests using a 1:4 dilution of antibody and 1:4 and 1:40 dilutions of antigen. Homologous CF titers were 1:64 or greater for ascitic fluids except for Marco (1:32), Flanders (1:8), and BFN 3187 (1:8). The viruses and their groupings are listed in Table 41 . Cross-reactions not previously reported were found between Piry antibody (1:4) and New Minto antigen; Mt. Elgon bat antibody (1:4-1:16) and rabies, Mokola, and BeAr 185559 antigens. The specificity of these reactions is not yet established. Tests with Yata, Keuraliba, Parry Creek, Kimberly, and Kununurra viruses are not yet done.

Table 41. Rhabdovirus Serological Classification

Group	Virus
VS	VS, Indiana
	Cocal
	VS, New Jersey
	Piry
	Chandipura
	Isfahan
Rabies	Rabies
	Mokola
	Lagos bat
	Duvenhage
	Obodhiang
	Kotonkan
Hart Park	Hart Park
	Flanders
Timbo	Timbo
	Chaco
Kwatta	Kwatta
	BeAn 157575
Mossuril	Mossuril
	Kamese
Sawgrass	Sawgrass
	New Minto
Ungrouped	Joinjakaka
	Barur
	Kern Canyon
	Oita 296
	BFN 3187
	Mt. Elgon bat
	BeAr 185559
	Porton S 1643
	Klamath
	Navarro
	Marco
	Yata
	Keuraliba
	Parry Creek
	Kimberly
	Kununurra

Electron microscope studies of Australian viruses. S.M.Buckley, M. Lipman, C.M.Woodroofe, and I. de Mattos. Two Australian viruses, ungrouped in serological studies, were characterized by E. M. Aus CG 668 from mosquitoes failed to multiply in BHK-21 cells. However, 8 days after passage from mouse brain through Singh's Aedes albopictus cell culture and back to mice, the agent was infective for BHK-21 cells. Rounding and granulation of BHK-21 cells was seen from 8 to 13 days after inoculation and the virus was passed 2 more times in BHK-21 cells, then examined by EM. Figure 7 shows a spherical 70 nm diameter particle in BHK-21 cells by thin-section electronmicroscopy. The particles contained an irregular nucleoid approximately 40 nm in diameter. There were no prominent projections. The particles were formed in cytoplasmic inclusions and were not seen in cytoplasmic vesicles. Morphogenetically, the particles were seen apparently escaping through the cell membrane. Aus CG668 appears to be an orbivirus.

Aus MI 19334, an isolate from ticks, infected BHK-21 cells with CPE in 7 days. Thin-section electron microscopy of infected BHK-21 cells revealed spherical particles, approximately 100 nm in diameter (Fig 8). The particles showed prominent outer projections, approximately 12 nm in length. There was an irregular dark core filling the membrane-bound particle. Virus was formed in cytoplasmic vesicles and fits the characteristics of a Bunyavirus.

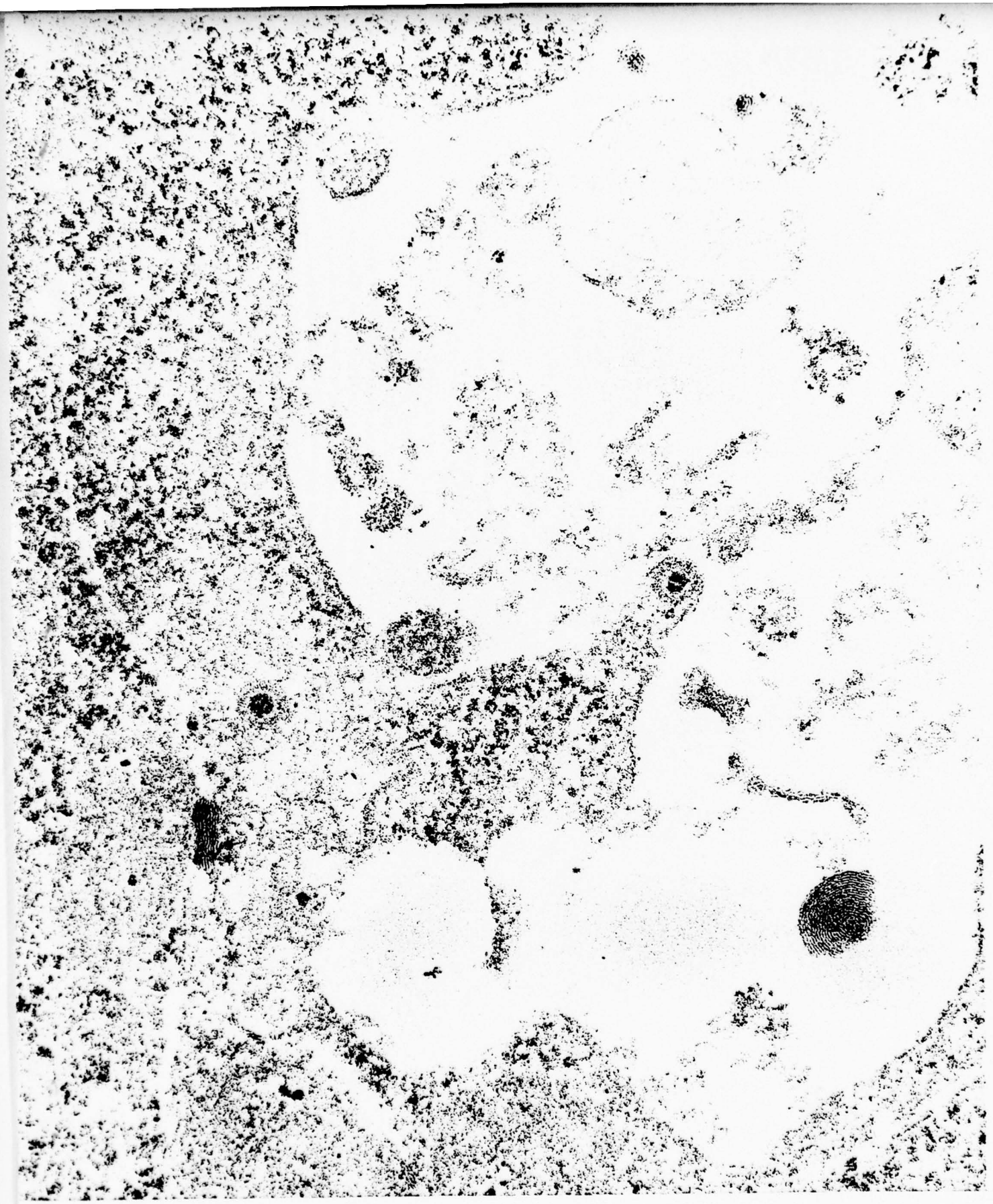


Figure 7. Thin-section electron microscopy of Aus GG668 virus. The spherical particles are 70 nm in diameter and contain an electron dense nucleoid characteristic of orbiviruses.

Figure 8. Thin-section electron microscopy of Aus MI 19334 virus in BHK-21 cells. Particles are approximately 100 nm in diameter and have prominent outer projections characteristic of Bunyaviruses.

III. Development of arbovirus techniques and virus models

Attachment of arboviruses to neural and non-neural cells in vitro*. A. Smith and G. Tignor. Two laboratory observations have led to the development of a model whereby the phenomenon of neurovirulence may be studied in vitro:

1). When the ability of several neurotropic arboviruses to infect mouse neuroblastoma cells was compared with that of serologically indistinguishable vaccine, avirulent or viscerotropic strains at several multiplicities of infection, it was found that these neuronal cells were highly sensitive to infection by neurovirulent strains, while they were either non-permissive or marginally so to the non-neurovirulent variants (see YARU 1976 Annual Report, p. 71-72).

2). Murine neuroblastoma cells in culture, which express both constitutive and differentiated, that is, neuronal, functions were highly sensitive to infection by rabies virus (1) which is essentially entirely neurotropic in its pathogenesis. Most other continuous cell lines do not support rabies growth to high titer.

Specific host and tissue tropisms of arboviruses both in vivo and in vitro have been documented but not explained. Since cell culture reactions uncomplicated by immunologic reactions now exist to study both the insect and vertebrate phases of the arbovirus cycle as well as the neuronal affinity of some arboviruses, the role of attachment in virus restriction and permissiveness is being investigated using variant strains of the same virus which express different tissue tropisms in vivo. The virus strains chosen for these studies are the mouse avirulent prototype Alphavirus, Sindbis (EgAr 339), and a neurovirulent variant of Sindbis, SaAr 86. The neurovirulent strain was isolated in South Africa from Culicine mosquitoes and was adapted to kill adult mice by intracerebral serial passage (2). One reason for choosing Sindbis virus as the model for this study is that immunosuppressed mice infected with the avirulent strain do not die (3), suggesting that its lack of virulence may be associated with a replicative event. The focus in this study is on adsorption in the replicative cycle because the possibility of intervening in viral replication at attachment may be somewhat less complex than at some other stages; it is likely that naturally occurring blocking agents can be found, and it is probable that host cells have not evolved a special set of receptors for viruses (4). The methods used to quantitate adsorption are based on enzyme kinetics and have been used in other virologic studies (5, 6). The cell lines used and their origin are:

CER	fibroblast-like, probably hamster
C6	rat glial
L929	mouse fibroblast
NT16	mouse neuron
N4	mouse neuron
N18	mouse neuron
Aedes albopictus	mosquito
Tadarida brasiliensis	bat lung

* This project was supported in part by NIH Grant 5-R01-AI 12541.

The studies with the Sindbis virus strains have focused on a) determining the kinetics of attachment process and b) determining the conditions for optimal attachment. A typical curve for the rates of attachment plotted against time is shown in Figure 9. Pre-cooled monolayer cultures on Lab-Tek slides were incubated at 4°C with radio-labelled virus at low input multiplicity. At the indicated times, the unadsorbed virus was removed and the cells washed. The pooled unadsorbed inoculum and wash fluid were used to determine the total unadsorbed virus. The attachment of Sindbis virus to empty glass slides was negligible. Attachment rate constants were calculated from the formula, $k=\ln (Vo/Vt)/nt$, where Vo=input virus concentration, Vt = unattached virus at time t, and n = the number of host cells per cm^3 of attachment medium. Equilibrium, that is, where the rate constant does not change with time, was reached for attachment of both strains to NT 16, L929, N4 and CER cell types by 3 hours. Thus, all subsequent attachment studies designated as "equilibrium" were performed for 3 hours at 4°C. It was also shown that rabies, eastern equine encephalitis, and St. Louis encephalitis virus reach equilibrium at an earlier time than the Sindbis strains (data not shown).

Table 42 illustrates the effect of varying virus diluents on the equilibrium attachment rates of the two Sindbis strains, grown in mammalian or insect cells, to CER cells; these rate constants reflect the affinity of binding. It can be seen that optimum conditions for attachment are dependent both on the virus strain and cell type used for propagation. Virus grown in mammalian cells appears to be more fastidious in its requirements for attachment.

Given the attachment data presented in Table 42, experiments were done to determine whether these results could be reproduced by infectivity assays (Table 43), realizing that infectivity assays measure steps in the replicative cycle in addition to attachment. The only correlation between the attachment and infectivity assay data was, in fact, the effect of $CaCl_2$ on EgAr 339. We conclude that the replicative events subsequent to attachment may have different requirements from those of attachment, as has been shown for the picornaviruses.

The rate of Sindbis virus attachment was strain-dependent when the two strains were propagated and attached to the same cell line (Table 42); the rates for single strain attached to different cell lines were next investigated. The equilibrium rate constants for EgAr 339 grown in CER cells and attached to CER and to C_6 cells are shown in Table 43. Optimal attachment to CER cells occurs with a high salt concentration and in the presence of $CaCl_2$. In contrast, attachment to C_6 cells shows little salt dependence, greater pH dependence and a decreased rate in the presence of fetal calf serum.

Table 44 shows that SaAr 86 attaches more efficiently in the presence of divalent cations and that attachment of both Sindbis strains is inhibited by the presence of fetal calf serum in the attachment diluent. In addition, the optimum conditions for EgAr 339 (Table 43) were shown not to be additive in their effect.

Although the drawbacks of studying attachment by infectivity assays have been previously pointed out, this technique is useful as a rapid and inexpensive method of determining the influence of the cell type used for virus propagation. The effect of the adsorption diluent on the titers of the two

Sindbis strains grown either in CER cells or in A. albopictus cells is shown in Table 46 . Salt concentration of the diluent has virtually no effect on A. albopictus-grown Sindbis, regardless of strain, whereas the titer of CER-grown EgAr 339 is influenced by salt concentration. The titers of both CER-grown Sindbis strains are only marginally influenced by pH, whereas low pH markedly decreases the infectivity of the A. albopictus-grown strains. In addition, the titers of the CER-grown strains were enhanced by the presence of $CaCl_2$ during adsorption, whereas divalent cations had little or no effect on the titers of A. albopictus-propagated strains.

These results now allow us to design experiments to enumerate the number of receptors per cell on several cell types for both of the Sindbis strains, and to determine whether different viruses or strains compete for the same receptor type. By enzyme sensitivity and receptor regeneration studies, the nature of the receptor can be defined. For instance, we already know that CER cell receptor activity for Sindbis virus strain Ar 339 is lost after protease treatment, and regeneration is complete after 3 hrs in nutritionally supplemented medium at both 37°C and 4°C, although there is a time lag for first appearance of receptor activity at 4°C. These techniques also allow us to do regeneration studies using metabolic inhibitors to determine whether new cellular synthesis is necessary for receptor appearance.

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Figure 9. Rates of attachment for SaAr 86 grown in N18 cells to C6 cells at 4°C vs. time

I = variation for 4 replicates

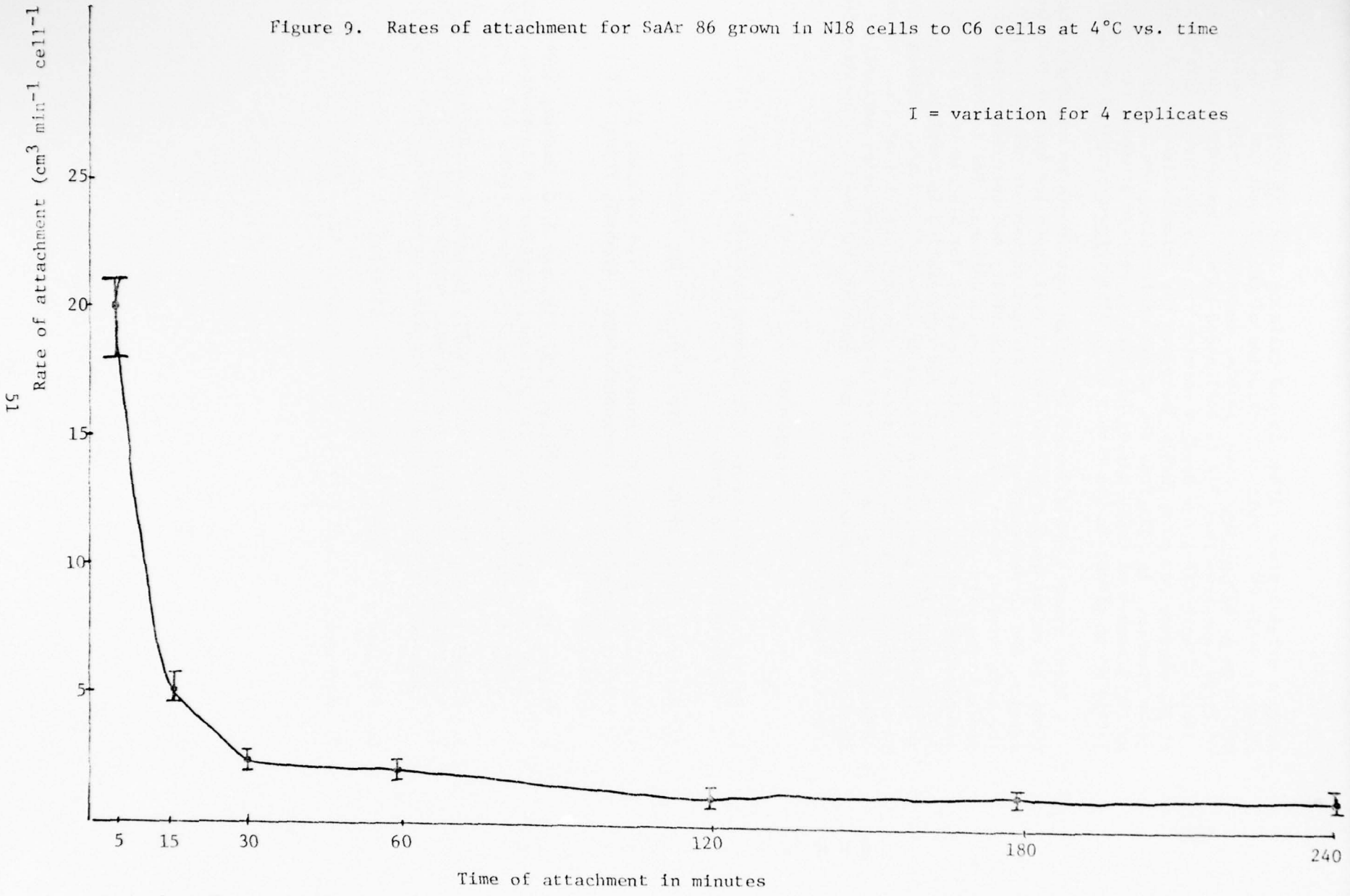

Table 42. Equilibrium rate constants ($cm^3\ min^{-1}\ cell^{-1} \times 10^{-9}$) for attachment of 2 Sindbis strains to CER cells at 4°C

Virus diluent[a]	SaAr 86 CER	Grown in *A. albopictus*	EgAr 339 CER	Grown in *A. albopictus*
PB, pH 7.2, 0.45M NaCl	62	1.6	35	0.0
PB, pH 7.2, 0.225M NaCl	58	1.2	18	3.5
PB, pH 7.2, 0.15M NaCl	40	2.1	1.0	3.7
PB, pH 7.2, 0.10M NaCl	7.5	3.8	0.6	2.9
PB, pH 7.2, 0.05M NaCl	6.1	5.3	1.5	5.4
PB, pH 7.2, 0.0093M NaCl	4.9	2.8	2.5	4.4
PB, 0.15M NaCl, pH 4.9	13	2.7	3.5	3.7
PB, 0.15M NaCl, pH 6.2	6.0	4.6	3.2	4.8
PB, 0.15M NaCl, pH 7.2	40	2.1	1.0	3.7
PB, 0.15M NaCl, pH 8.1	71	3.4	5.2	3.5
PB, 0.15M NaCl, pH 7.2 with 10% FCS	19		3.5	
PB, 0.15M NaCl, pH 7.2 with 9×10^{-4}M $CaCl_2$	54	4.7	12	2.8
PB, 0.15M NaCl, pH 7.2 with 9×10^{-4}M $MgCl_2$	54	2.1	0.8	2.8
PB, 0.41M NaCl, pH 7.9	34			
PB, 0.41M NaCl, pH 7.9 with 4×10^{-4}M $CaCl_2$	37			
PB, 0.41M NaCl, pH 7.9 with 4×10^{-4}M $CaCl_2$ 7×10^{-4}M $MgCl_2$	46			

[a] Sorensen's phosphate buffers (7); cultures were washed with buffer used for attachment.

Table 43. Comparison of attachment with infectivity assays of 2 Sindbis strains in CER cells

Virus diluent[b]	Strain[a] SaAr 86		EgAr 339	
	radio-labelled virus %attached[c]	$\log_{10}$ PFU/ml[d]	radio-labelled virus %attached[c]	$\log_{10}$ PFU/ml[d]
PB, pH 7.2, 0.45M NaCl	65.7 ± 4.5	5.9	69.7 ± 3.9	6.2
PB, pH 7.2, 0.225M NaCl	63.3 ± 4.1	5.9	46.3 ± 3.0	6.7
PB, pH 7.2, 0.15M NaCl	49.4 ± 6.4	5.9	3.3 ± 2.1	6.7
PB, pH 7.2, 0.10M NaCl	12.0 ± 4.4	6.0	2.0 ± 4.2	6.8
PB, pH 7.2, 0.05M NaCl	9.9 ± 3.8	6.4	5.1 ± 6.6	7.5
PB, pH 7.2, 0.0093M NaCl	8.0 ± 4.5	6.4	8.1 ± 4.5	7.6
PB, 0.15M NaCl, pH 4.9	19.4 ± 4.3	5.7	11.3 ± 2.9	6.0
PB, 0.15M NaCl, pH 6.2	9.8 ± 2.9	6.1	10.4 ± 3.4	N.D.
PB, 0.15M NaCl, pH 7.2	49.4 ± 6.4	5.9	3.3 ± 2.1	6.7
PB, 0.15M NaCl, pH 8.1	70.4 ± 4.3	6.0	16.3 ± 3.0	6.4
PB, pH 7.2, 0.15M NaCl with 9×10^{-4}M $CaCl_2$	60.3 ± 6.0	6.8	32.6 ± 4.5	8.1
PB, pH 7.2, 0.15M NaCl with 9×10^{-4}M $MgCl_2$	60.2 ± 3.2	5.9	2.8 ± 4.0	7.3

(a) Both strains grown in CER cells.

(b) Sorensen's phosphate buffers; cultures were washed with buffer used for attachment.

(c) Attachment at 4°C for 3 hours.

(d) Adsorption at 4°C for 1 hour, replicate titrations resulted in variation <0.4 $\log_{10}$ OFY/ml.

N.D. = Not done.

Table 44 . Equilibrium rate constants (cm^3 min^{-1} $cell^{-1}$ X 10^{-9}) for Sindbis (EgAr-339) attachment to 2 cell lines at 4°C [a]

Virus diluent [b]	Attachment to CER cells	Attachment to C6 cells
PB, pH 7.2, 0.45M NaCl	35	9.5
PB, pH 7.2, 0.225M NaCl	18	8.7
PB, pH 7.2, 0.15M NaCl	1.0	9.0
PB, pH 7.2, 0.10M NaCl	0.6	8.3
PB, 0.15M NaCl, pH 6.2	3.2	2.6
PB, 0.15M NaCl, pH 7.2	1.0	9.0
PB, 0.15M NaCl, pH 8.1	5.2	8.5
PB, 0.15M NaCl, pH 7.2 with 10% FCS	3.5	3.5
PB, 0.15M NaCl, pH 7.2 with 9 X 10^{-4}M $CaCl_2$	12	8.5
PB, 0.15M NaCl, pH 7.2 with 9 X 10^{-4}M $MgCl_2$	0.8	8.5

[a] Grown in CER cells.

[b] Sorensen's phosphate buffers; cultures were washed with attachment buffer.

Table 45. Equilibrium rate constants ($cm^3\ min^{-1} cell^{-1} X10^{-9}$) for Sindbis attachment at 4°C [a]

Virus diluent [b]	Virus strain				
	SaAr 86				EgAr-339
	CER	Attached to TB [c]	Attached to N18	Attached to C6	Attached to C6
PB, pH 7.9, 0.41M NaCl	34	16	140	28	9.6
PB, pH 7.9, 0.41M NaCl with 4 X 10^{-4}M $CaCl_2$	37	19	160	34	9.4
PB, pH 7.9, 0.41M NaCl with 4 X 10^{-4}M $CaCl_2$, 7 X 10^{-4}M $MgCl_2$	46	24	200	46	9.2
PB, pH 7.2, 0.15M NaCl	40	16	190	40	9.0
PB, pH 7.2, 0.15M NaCl with 10% FCS	19	8.5	90	22	3.5

[a] Both strains grown in CER cells.

[b] Sorensen's phosphate buffers; cultures were washed with attachment buffer.

[c] Tadarida brasiliensis (bat lung cells).

Table 46. Comparison of infectivity titers[a] in CER cells for 2 strains of Sindbis grown in mammalian and insect cell cultures[b].

Virus diluent[c]	SaAr 86 Grown in		EgAr 339 Grown in	
	CER	A. albopictus	CER	A. albopictus
PB, pH 7.2, 0.45 NaCl	5.9	5.2	6.2	3.9
PB, pH 7.2, 0.225M NaCl	5.9	5.0	6.7	3.9
PB, pH 7.2, 0.15 NaCl	5.9	5.0	6.7	3.9
PB, pH 7.2, 0.10M NaCl	6.0	5.2	6.8	3.9
PB, pH 7.2, 0.05M NaCl	6.4	5.3	7.5	4.2
PB, pH 7.2, 0.0093M NaCl	6.4	5.2	7.6	4.5
PB, 0.15M NaCl, pH 4.9	5.7	3.6	6.0	<1.3
PB, 0.15M NaCl, pH 6.2	6.1	4.3	-	3.8
PB, 0.15M NaCl, pH 7.2	5.9	5.0	6.7	3.9
PB, 0.15M NaCl, pH 8.1	6.0	4.6	6.4	3.8
PB, 0.15M NaCl, pH 7.2 with 9 X 10^{-4}M $CaCl_2$	6.8	4.8	8.1	3.8
PB, 0.15M NaCl, pH 7.2 with 9 X 10^{-4}M $MgCl_2$	5.9	4.6	7.3	3.8

[a] Log_{10} PFU/ml; replicate titrations resulted in variation <0.4 log_{10} PFU

[b] Adsorption at 4°C for 1 hour.

[c] Sorensen's phosphate buffers; cultures were washed with attachment buffer.

High salt concentration increases the pH range of dengue HA antigens. B. Beaty and R. Kowalski. Dengue types 1, 2, 3, and 4 antigens from sucrose-acetone extracted mouse brain were tested for their pH range and titer at NaCl concentrations in the phosphate buffers over a range from .15M to .8M. It had previously been shown with antigens of the family Bunyaviridae that high salt concentrations enhanced the titer of HA with goose cells.

The titers of dengue antigens were not enhanced; the pH range, however, was enhanced, especially at 0.8M NaCl (Table 47). HI tests were carried out at each salt concentration over the maximum possible pH range. The HA was specific in each case. Neither homologous nor heterologous antibody titers varied greatly with either pH or salt concentration.

The mechanism of increased HA titers with Bunyaviruses and broadened HA pH range with flaviviruses is not yet known.

Table 47 . The increase in pH range of dengue virus HA with high NaCl concentration.

Dengue 1

	NaCl Concentration				
pH	0.15M	0.25	0.4	0.6	0.8
5.75	10240	10240	10240	20480	20480
6.0	10240	10240	10240	20480	20480
6.2	5120	10240	10240	20480	10240
6.4	1280	640	2560	5120	10240
6.6	0	0	0	0	1280
6.8	0	0	0	0	0
7.0	0	0	0	0	0

Dengue 2

pH	0.15	0.25	0.4	0.6	0.8
5.75	20	0	20	80	40
6.0	40	40	40	80	20
6.2	160	160	80	160	80
6.4	0	80	160	160	160
6.6	0	0	0	0	80
6.8	0	0	0	0	0
7.0	0	0	0	0	0

Table 47 cont.

Dengue 3

pH	0.15	0.25	0.4	0.6	0.8
5.75	1280	1280	1280	1280	640
6.0	1280	1280	1280	640	640
6.2	1280	1280	1280	1280	640
6.4	1280	1280	1280	1280	1280
6.6	1280	640	640	640	640
6.8	80	320	320	160	640
7.0	0	0	80	20	160

Dengue 4

pH	0.15	0.25	0.4	0.6	0.8
5.75	640	1280	640	640	640
6.0	640	640	640	640	640
6.2	320	640	320	640	640
6.4	0	80	80	160	320
6.6	0	0	0	40	80
6.8	0	0	0	0	40
7.0	0	0	0	0	0

Hemagglutination of WEE virus from infected Culiseta melanura mosquitoes. S. Hildreth with R. Shope. Culiseta melanura were inoculated intrathoracically with 50sm IC LD_{50} of either the Connecticut 72-666 or the California FMS 813 strains of WEE virus. The mosquitoes were held for 8 days, then sonicated in borate-saline buffer and the tissues extracted by the sucrose-acetone method. The 72-666 pool contained 2 males and the FMS 813 pool contained 16 females. Both CF and HA antigens were detected. The HA antigens were shown to be specific in the HI test using 0.4M NaCl. The titers of both antigen were enhanced by increasing the NaCl concentration of the phosphate buffer system as shown in Table 48 although the specificity of the very high titers at .8M NaCl pH 5.75 and 6.0 was not tested.

Table 48 . Titers at varying pH and molarity; WEE antigens from mosquito tissues

Molarity	pH	Strain 72-666	Strain FMS 813
.15 NaCl	5.75	32	8
	6.0	32	16
	6.2	16	16
	6.4	0	8
	6.6	0	8
	6.8	0	0
.4 NaCl	5.75	64	64
	6.0	64	32
	6.2	0	16
	6.4	0	8
	6.6	0	4
	6.8	0	0
.8 NaCl	5.75	>4096	>4096
	6.0	>4096	256
	6.2	256	128
	6.4	64	64
	6.6	0	8
	6.8	0	8

Evaluation of serological techniques for diagnosis of CHF-C virus infection. J. Casals and J.E. Vasilenko. Serological diagnosis of current cases of CHF in the USSR and Bulgaria has been done mainly by CF and agar gel diffusion precipitation (AGDP) tests; it has been noted (Karinskaya et al., Miscellaneous Publications, 9: 142-144, 1974) that antibodies persist for at least 3 or 4 years after onset in persons who had a clinical case of CHF, but with considerable loss in titer. Sero-epidemiological surveys are hindered by the difficulty in carrying out neutralization tests, either in mice by intracerebral route of inoculation or by plaque reduction; the test is not sensitive and sera have non-specific neutralizing substances that require elimination prior to a test.

While awaiting development of a sensitive and specific neutralization test, immunofluorescence and HI tests should be considered as means to conduct seroepidemiological surveys; the need for the latter is pressing insofar as there still is a controversy as to whether subclinical or undiagnosable infections occur with this virus.

Table 49. Serological tests with sera from human survivors of CHF, collected in 1973.

Case No.	Bled, years from onset	Test			
		Sofia AGDP	YARU		
			CF	IF	HI
1	19	+	8	±	20
2	19	+	8	+	40
3	19	+	16	+	40
4	19	+	16	+	80
5	19	+	0	0	40
6	18	-	0	0	0
7	18	+	8	+	20
8	18	+	8	+	80
9	9	-	4	+	20
10	9	+	16	+	20
11	8	+	8	+	40
12	8	-	4	+	20
13	8	-	16	+	20
14	8	-	0	0	20
15	8	+	16	+	40
16	6	-	4	+	10
17	6	+	0	0	0
18	6	+	8	+	40
19	6	+	32	64	20
20	5	+	4	+	80
21	5	+	8	+	80
22	4	-	0	0	10
23	4	-	16	+	10
24	4	+	4	+	20
25	3	+	8	+	160
26	2	+	8	32	20
27	2	+	16	+	40
28	1	+	16	+	20
29	?	+	32+	+	160

AGDP, positive or negative: dilution used not known.

CF, reciprocal of serum titer, lowest dilution 1:4.

IF, only dilution 1:4 used, except where the reciprocal of titer is given.

HI, reciprocal of serum titer, lowest dilution 1:10.

Table 50 . Serological tests with sera from persons vaccinated against CHF virus

Individual No.	Bled, years after vaccination	Test			
		Sofia, AGDP	YARU		
			CF	IF	HI
30	3	+	NS (1:8)	0	0
31	3	+	32	0	0
32	2	-	NS (1:16)	0	0
33	2		0	0	0
34	2	+	8	0	0
35	2		8	+	0
36	2	+	0	0	0
37	2	+	16	+	0
38	1 or less	+	NS (1:16)	?	0
39	"	+	NS (1:8)	0	0
40	"	+	16	+	0
41	"	+	32	+	80
42	"	+		0	0
43	"	+		+	20
44	"	+	NS (1:16)	0	20
45	"	+		0	0
46	"	+	16	+	0
47	"	+		0	0
48	"	+		+	0
49	"	+		+	40
50	"	+		+	40

See Table 49.

NS (1:8), serum at dilution 1:8 gave positive reaction with normal brain antigen.

Dr. V. E. Vasilenko, Sofia, Bulgaria, submitted to YARU 29 sera from persons who had recovered from CHF in Bulgaria; and 21 from other individuals who had been vaccinated against CHF. The course of vaccination and the nature of the vaccine has not been ascertained at this writing; most likely, the vaccine is prepared from infected mouse brain tissue and is partially purified by centrifugation and precipitation by protamine sulphate, and inactivated by formalin.

The sera had been tested by CF and AGDP tests by Dr. Vasilenko; on repeating the former tests in this laboratory, it was found that the titers reported by Dr. Vasilenko were much higher than those observed at YARU and, particularly among vaccinees, many gave non-specific reactions with normal mouse brain tissue antigens.

The sera were tested at YARU by CF, HI and the immunofluorescence indirect test (IF); the combined results are shown in Tables 49 and 50 .

As Table 49 indicates, antibodies were detected by all tests used for as long as 19 years after onset; probably all or most individuals resided in endemic foci where they had acquired their disease, therefore the possibility of repeated exposures after their illness, capable of acting as antibody boosters, cannot be ruled out.

In Table 50 , the reactions given by sera from vaccinees are shown. Five of 14 sera thus far tested by CF gave non-specific reactions most likely due to the fact that mouse brain tissue was the vaccine substrate, as well as the source of antigen; it is conceivable that the same mechianism could account for the almost complete positiveness in the AGDP test. By contrast, as well as in contrast with the result with sera from patients, fewer of the vaccinees had antibodies detectable by IF and fewer still by HI.

The possible different reactivity of antibodies following inoculation of inactivated virus and following infection is being investigated.

Polyacrylamide gel electrophoresis of viral RNA's and proteins. D. Knudson, R. Shope, and A. Main. The polyacrylamide gel electrophoresis (PAGE) technique was utilized to determine relative migration distances of segments of dsRNA of Reoviridae. Initial attempts in slab gels with ds RNA of reovirus type 3 indicate feasibility of the project. Twenty-seven orbiviruses of the Kemerovo group have been grown in BHK-21 cells and titered preparatory to PAGE analysis of their RNA's. The patterns will be compared to determine which segments differ, and hopefully to develop a characterization technique to complement the serology. The results should offer a more critical means than serology of determining evolutionary pattern and closeness of relationship, especially with the many Kemerovo group viruses from Ixodes uriae, multiple serotypes of which occur in the same tick populations.

LaCrosse-Snowshoe hare virus RNA-reassortent pathogenicity and serology. R. Shope. In collaboration with J. Gentsch, L. Wynne, J. Clewley and D. Bishop of the University of Alabama, RNA-reassortents of LaCrosse and snowshoe hare virus were studied. Reassortents and parent viruses were supplied as infected BHK-21 cell fluids. The pathogenic potentials of the wild-type viruses (inoculated either singly or together) and six of the LAC-SSH

recombinants were determined by intraperitoneal inoculation of groups of five 3-week-old mice, using two dilutions of the viruses. Groups of two or three adult hamsters and of two adult guinea pigs were also used for similar tests. The results, in terms of mortality rates and survival times, are shown in Table 51 . None of the guinea pigs, all of which were inoculated intraperitoneally with 0.1 ml of a 10^{-1} dilution of virus, died during 21 days of observation, although each received between 2.6×10^6 and 1.8×10^5 PFU of virus and most, by CF and HI tests seroconverted (see Table 52).

The inoculated mice received between 2.6×10^7 and 1.8×10^4 PFU of virus (Table 51). All died between 2 and 6 days post-inoculation. However none of the groups of mice that received the putative recombinant viruses died significantly earlier than those receiving the one or two wild-type viruses. Some of the mice that were inoculated with the putative recombinants did, however, survive significantly longer than those that received the wild-type viruses (e.g., the recombinant derived from LAC II-4 X SSH I-1). Although no correlation has yet been attempted for the ratio PFU titer/mean lethal dose for recombinants with those of the wild-type viruses, these results suggest that certain recombinants may allow mice an increased survival time compared to those receiving the wildtype progenitors.

The hamsters that were inoculated subcutaneously received between 2.6×10^6 and 1.8×10^5 PFU of virus. One of the two hamsters inoculated with wild-type progenitor LAC virus died on day 13; the two inoculated with wild-type progenitor SSH virus died on days 8 and 16. Two of 14 hamsters that received the recombinant viruses died (LAC II-5 X SSH I-3 died on day 11; LAC II-5 X SSH I-3 died on day 13); the rest survived.

The results of CF and HI analyses using test SSH and LAC antigens and the 21-day sera obtained from the animals that survived the virus inoculation are shown in Table 52 .

The individual sera of the guinea pigs that received wild-type LAC virus although clearly cross-reactive by both CF and HI tests could be typed as containing LAC antibodies. The individual sera of the guinea pigs that received wild-type SSH virus were less cross-reactive than those obtained for LAC and clearly contained SSH antibodies. A LAC CF response and an ambivalent HI response were obtained for the sera recovered from the guinea pigs that received both viruses.

No hamsters survived the wild-type SSH virus infections, however, the one hamster that survived the wild-type LAC infection gave a serum sample that, by CF and HI tests, as in the corresponding guinea pig serum tests, was compatible with it containing LAC antibodies. The hamster that survived the dual wild-type virus infections gave a serum sample that, by CF and HI tests, contained antibodies directed against SSH virus.

The reassortent virus obtained from the LAC II-3 X SSH I-1 cross gave an LAC-type HI response and an SSH-type CF response for both the guinea pig and hamster sera. All of the other recombinants gave LAC-type CF and HI responses for sera obtained from guinea pigs or the available hamsters.

The serological results obtained for the LAC II-5 X SSH I-3 reassortent agree well with the observation of the University of Alabama group that the

reassortent has an SSH/LAC/LAC genome and an LAC-type N protein, and could be interpreted as indicating that the LAC M RNA and/or S RNA provide LAC gene products as assayed by the HI and CF tests. The results obtained for the LAC II-4 X SSH I-1 reassortent,which has an SSH/LAC/SSH genome and an SSH-type N protein, do not fit the interpretation that the S RNA codes for gene products that were measured by the CF or HI tests. Although that may be true for that particular reassortent, the Alabama group has found that virion protein analyses of the LAC II-3 X SSH I-1 reassortent indicate that it has an SSH-type N protein. Although no fingerprint analyses have been undertaken for the LAC II-3 X SSH I-1 recombinant, the serological analyses indicate an SSH-type CF response and an LAC-type HI response, suggesting that for this recombinant SSH L and/or S RNA code for the gene products that were responsible for generating the SSH-type CF responses.

Although indicative, due to the cross-reactive nature of the LAC CF and HI antibody responses (and, to a lesser extent, those obtained for SSH virus), the serological results are not conclusive. Further analyses with individual viral antigens, as well as more refined immunological tools, will be needed to establish what antigens are involved in the HI and CF tests.

It is noteworthy that some of the groups of cohabitating hamsters that received a particular reassortent virus included one or two hamsters that did not seroconvert. Although the animals were only kept for 21 days post-inoculation, the lack of seroconversion might mean that no direct transmission of the virus between cage mates occurred. Further experiments involving cohabitating uninoculated and inoculated mice and other animals will be used in future pathogenicity tests to investigate this point.

Table 51. Intraperitoneal pathogenicity in 3-week-old mice of LAC, SSH, and putative LAC-SSH reassortents[a].

Virus	Inoculum titer (PFU/ml)	Average Survival time (days)	
		inoculum: 0.1 ml of 10° dilution	Inoculum: 0.1 ml of 10° dilution
LAC (wild type)	1.2×10^8	2.6 ± 0.49	3.0 ± 0.0
SSH (wild type)	3.4×10^7	2.2 ± 0.40	3.0 ± 0.0
LAC X SSH (wild type)	1.5×10^8	3.0 ± 0.0	3.0 ± 0.0
Recombinant cross			
LAC II-3 X SSH I-1	1.7×10^8	3.0 ± 0.0	4.0 ± 0.0
LAC II-3 X SSH I-3	2.6×10^8	2.6 ± 0.49	2.8 ± 0.57
LAC II-4 X SSH I-1	2.3×10^8	4.0 ± 0.0[b]	5.8 ± 0.57[b]
LAC II-4 X SSH I-3	2.2×10^8	4.0 ± 0.0[b]	4.2 ± 0.04[b]
LAC II-5 X SSH I-1	1.8×10^7	5.2 ± 0.40[b]	5.8 ± 0.57[b]
LAC II-5 X SSH I-3	1.6×10^8	3.0 ± 0.0	3.0 ± 0.0

[a]Groups of five 3-week-old mice were inoculated intraperitoneally with two dilutions of the wild-type virus stocks or an equal-volume mixture of the wild-type viruses and observed until death occurred. Virus stocks obtained from the cloned reassortents from the indicated mutant virus crosses were similarly used to inoculate mice. All inoculated mice died, and the average survival times are given.

[b]The average survival time, which differed from that of either parent wild type, is significant by Student's t-test. $P<0.001$.

Table 52. Serological analyses of guinea pigs and the surviving hamsters that were inoculated with LAC virus and/or SSH virus, or the LAC-SSH reassortents.[a]

Inoculum	Test antigen	Guinea pig CF	Guinea pig HI	Serotype (CF-HI)	Hamster CF	Hamster HI	Serotype (CF-HI)
LAC (wild type)	LAC	32	40	LAC-LAC	32	10	LAC-LAC
	SSH	32	20		32	<10	
SSH (wild type)	LAC	8	10	SSH-SSH	NS[b]	NS	
	SSH	128	160		NS	NS	
LAC x SSH (wild types)	LAC	64	20	LAC?	32	20	SSH-SSH
	SSH	32	20		128	80	
Reassortent							
LAC II-3 x SSH I-1	LAC	32	80	SSH-LAC	16	80	SSH-LAC
	SSH	64	40		64	20	
LAC II-3 x SSH I-3	LAC	32	40	LAC-LAC	128	80	LAC-LAC
	SSH	16	20		64	80	
LAC II-4 x SSH I-1	LAC	128	40	LAC-LAC	128	80	LAC-LAC
	SSH	64	20		128	40	
LAC II-4 x SSH I-3	LAC	64	80	LAC-LAC	32	40	LAC-LAC[c]
	SSH	64	40		32	20	
LAC II-5 x SSH I-1	LAC	32	20	LAC-LAC	64	40	LAC-LAC[d]
	SSH	32	10		64	20	
LAC II-5 x SSH I-3	LAC	64	80	LAC-LAC	NR[e]	NR	
	SSH	32	20		NR	NR	

[a] CF and HI antibody responses were determined in sera of surviving animals by using SSH or LAC test antigens.
The guinea pig and hamster sera were tested on an individual basis with the alternate sera giving the same results as those shown in these assays, except when no HI or CF response was detected, suggesting that the animal was not infected.

[b] NS, No survivors.

[c] One of two animals not infected, as judged by no CF or HI response.

[d] Two of three animals not infected, as judged by no CF or HI response.

[e] NR, No response.

IV. Serologic Surveys

Lassa virus antibodies in hospital personnel in Liberia. J. D. Frame and J. Casals. Serum samples were obtained early in 1977 from personnel in several hospitals in Liberia. Past observations had shown the presence of Lassa fever and/or antibodies in the area of Africa in which Sierra Leone, Guinea and Liberia come together; for this reason hospitals were chosen in Liberia for this survey which were in the north-eastern section of the country. The purpose of the survey was two-fold: to determine the extent of Lassa virus infection using the hospital personnel as easily accessible experimentative populations of the respective areas; and to locate immune persons who may be interested in supplying plasma by plasmapheresis and thus establish a stock of valuable antibody.

The serological test used for the survey was the indirect immunofluoresnce (IF), employing as antigen Vero cells infected with the virus (later inactivated); drops of the cells were deposited on teflon-coated microscope slides having 12 circular areas 5mm in diameter uncoated. The slides were generously supplied by Drs. K. M. Johnson, P. Webb and H. Wulff, CDC, Atlanta, Georgia. An incident light microscope was used, set for blue fluorescence and equipped with a Xenon Osram 200 lamp.

Sera were tested only at dilution 1:4 and classified as positive, negative and questionable; a number of the questionable sera on retesting at dilution 1:2 or undiluted became clearly positive.

In addition to 482 sera from Liberia, were tested 26 from Cameroon and 81 from Benin (formerly Dahomey); the combined results are given in Table 53, and the approximate location of the hospitals surveyed is shown in Figure 10.

It is interesting to notice that the proportion of positives is highest nearer to the endemic area the hospitals surveyed were; thus at Foya, 22% of 41 persons tested were positive as well as 12% of 97 individuals tested at Zorzor. In contrast, none of 27 tested at Elwa was clearly positive.

It is also to be remarked that positive sera were observed for the first recorded time in Cameroon and Benin.

Investigations under the direction of Dr. Frame are being continued, with the purpose to determine by medical history, for as many of the positive donors as can be reached, whether there is clinical evidence of Lassa fever among them.

Serosurvey of villages of Senegal River.* W. G. Downs and G. Roze. One hundred serum specimens were selected as an intentionally biased sample, equal numbers of males and females and equal numbers from 5 Senegal River villages, limited to those between 10 and 25 years of age.

*This project was supported by a contract from AID (Afr/AID-C-1259); it was made possible by, and utilized resources of the Arbovirus Reference Center.

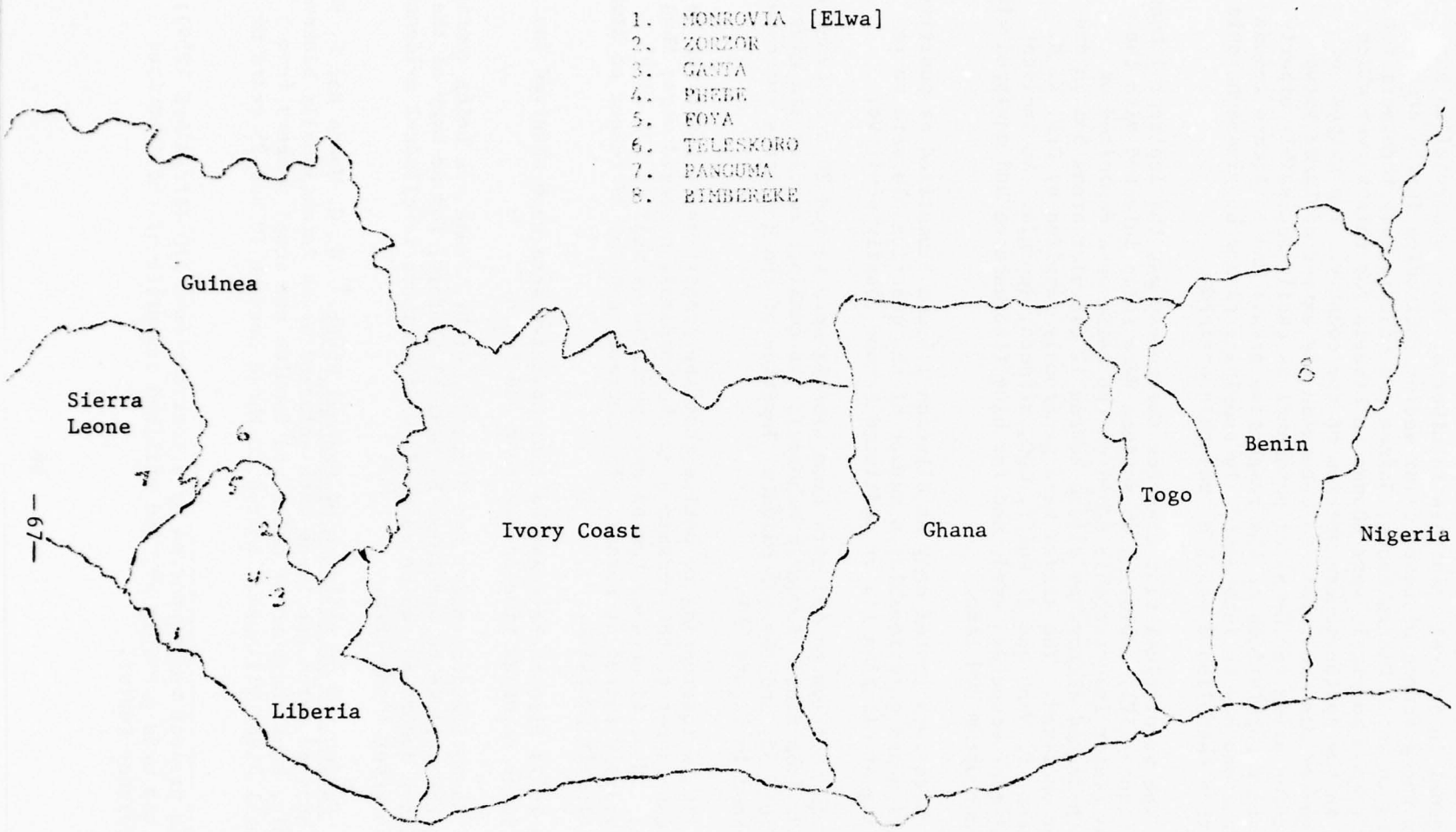

Figure 10, location of Liberian hospitals whose personnel were surveyed for Lassa virus antibodies.

Table 53 . Immunofluorescence test: survey for Lassa virus antibodies, Africa

		Number	Positive (%)	?	(%)	Negative (%)
Liberia	Foya	41	9 (22)	1	(2.5)	31 (75.5)
	Zorzor	97	12 (12.4)	5	(5)	80 (82)
	Phebe	253	25 (10)	19	(7.4)	209 (83)
	Ganta	64	4 (6.2)	5	(7.8)	55 (86)
	Elwa	27	0 (0)	4	(14)	23 (86)
Cameroon	-	26	1	0		25
Benin	Bimbereke	81	2	1		78

The age selection bias is intentional. Individuals are old enough to have acquired infections, yet young enough possibly to avoid being saturated by infections with several related viruses in the alphavirus and flavivirus groups, particularly. It is in this group that one may hope to encounter individuals whose serological pattern in HI testing may permit a guess as to the causative virus. Specific virus neutralization tests to define further the virus specific reactor have not yet been carried out.

In the alphavirus group, there were reactions as follows:

Virus	Number positive / Number tested	Range of titer levels
chikungunya	2/100	1:80 and 1:640
Middelburg	0/100	----------
Ndumu	1/100	1:20
Semliki Forest	2/100	1:10 and 1:40
Sindbis	29/100	1:10 and 1:160

The Sindbis positive reactors have 15 reacting at 1:10; 7 at 1:20; 6 at 1:40 and 1 at 1:160. These probably represent specific Sindbis antibody.

The Semliki Forest reactor at 1:40 reacts with chikungunya at 1:640 and the reactor at 1:10 reacts with chikungunya at 1:80. Both infections are almost certainly chikungunya virus infections. The single Ndumu reactor, at 1:20, was reactive to all the other alphavirus hemagglutins,

including chikungunya at 1:640, and almost certainly represents a non-specific alphavirus serological cross reaction, induced by infection with more than one alphavirus.

In the flavivirus group, the picture is much more complicated.

Virus	Number positive / Number tested	Range of antibody levels
Banzi	68/100	1:10 - 1:640
Dengue I	35/100	1:10 - 1:80
Dengue II	38/100	1:10 - 1:80
Wesselsbron	73/100	1:10 - 1:640
West Nile	54/100	1:10 - 1:160
Yellow Fever	48/100	1:10 - 1:160
Zika	31/100	1:10 - 1:320
Kadam	47/100	1:10 - 1:160
Dakar Bat	31/100	1:10 - 1:80
Israel Turkey Meningoencephalitis	52/100	1:10 - 1:640

At casual glance, there appears to be the expected finding of a hopelessly entangled flavivirus HI cross-reactivity. This would indicate infection with one or more flaviviruses, with the specific viruses not determinable, probably not even by neutralization test.

Sera which reacted to only one or two antigens were selected out, and 8 Wesselsbron reactors thus located. Three of these reacted only to Wesselsbron antigen and at 1:10 dilution only. Three reacted with Wesselsbron 1:10 and Banzi 1:10; one reacted with Wesselsbron 1:20, Banzi 1:10 and one reacted with Wesselsbron 1:10 and West Nile 1:10. A Wesselsbron neutralization test will be carried out.

The remainder of the sera are broadly cross-reactive and interpretation to specific levels is not possible.

The 100 sera were also tested and found negative by HI with Sicilian sandfly fever antigen.

Survey for antibodies against selected arboviruses in sera from Brazil. I. Mattos and J. Casals. Activity of arboviruses in the southern most part of Brazil, State of Rio Grande do Sul, has not been investigated heretofore to any large extent. A number of sera were obtained from residents from two localities in the State, 63 sera from Uruguaiana and 29 from Pelotas; in addition and as a contrasting situation, 69 sera were obtained from residents of Rio de Janeiro. The sera from Uruguaiana were collected in 1975, the others in 1977. Most of the sera were from adult males; due to the small numbers involved no attempt can be made to analyze the results in terms of sex, age or other parameters.

All sera were tested by HI and, subsequently, a few selected sera by neutralization test. The following antigens, in 8 unit amounts, were used with all sera:

Group A (alphavirus), VEE, WEE, EEE, Aura and Mayaro; group B (flavivirus), Aroa, Ilheus, Rocio (San Paulo encephalitis), dengue type 2, yellow fever, Bussuquara, and St. Louis encephalitis; group C, Oriboca; and group Bunyamwera, Guaroa and Maquari. In addition, the sera from Uruguaiana were tested against Marituba.

A summary of the results is given in Tables 54 and 55 . Table 54 indicates the overall reactivity of the sera against any antigen of a group; all positive sera were positive against a group B antigen, possibly also to antigens of other groups. It is of interest to notice that hardly any sera from Uruguaiana and Pelotas reacted with group A antigens while a large proportion, 21 of 69, from Rio de Janeiro reacted with antigens of that group; in fact, most reactions with the latter sera were with Mayaro antigen. Reactivity with group B antigens was marked in the three localities with 52 of 63 being positive in Uruguaiana, 18 of 27 in Pelotas, and 57 of 69 in Rio de Janeiro. The number of sera positive for either group C or Bunyamwera group was small and at low titers.

Table 55 gives a break down with group B antigens with which all positive sera reacted. The serological diagnosis within group B is presumptive, applies only to the viruses included in the survey and is based on the observation of a 2-fold or greater titer with an antigen than with the next highest titer with another antigen. Under superinfection are placed those sera that reacted broadly and at relatively high titers with 2 or more antigens, with usually similar titers with most antigens. In the questionable column are placed sera that reacted with minimum titers, usually 1:10 or 1:20, and with two or more antigens.

From an examination of Table 55 it appears that the sera from Uruguaiana had HI antibodies almost exclusively diagnosed as SLE; in contrast, neither Rio de Janeiro nor Pelotas sera show a definite specific trend, with mainly low titered reaction among the few positive only for Aroa, and many in Rio de Janeiro, 36 of 69, being positive with the typical broad reaction of superinfection.

Table 56 lists examples of results given by selected sera from Uruguaiana which were diagnosed as SLE by HI, with one serum, #20, which gave a characteristic superinfection-type response.

Since the presence of SLE virus has not been reported in Rio Grande do Sul, an effort was made to determine whether the sera that were positive by HI against this virus would also be positive by neutralization test. Unfortunately, there were difficulties with the plaque reduction tests with the viruses used, SLE, Bussuquara, Aroa, and Ilheus, which made the results erratic and uninterpretable. Attempts to test sera by i.c. mouse neutralization test were curtailed by the small amounts of serum available. An abridged type of test could only be done, with the three viruses listed in Table 56 used at a dilution only -- which turned out to be between 100 and 1000 i.c. LD_{50} -- and with the sera used in dilution 1:2. The results of these rather severe tests done in newborn mice, fail to show complete protection by any serum against any virus.

However, whereas not a single mouse was protected against Bussuquara virus and only one serum, #56, gave partial protection against Aroa, 5 sera of 13 tested - #5, 6, 10, 20, and 56 -- gave partial protection against St. Louis virus.

It is still premature to claim that SLE virus is responsible for the antibodies found in a number of Uruguaiana residents; it is not unreasonable, however, to entertain this notion in view of our results and of the fact that SLE virus has been isolated in other areas of Brazil and also in northern Argentina.

Table 54 . Hemagglutination-inhibition test: survey for arbovirus antibodies in Brazil

Origin and number of sera	Positive, any Antigen	Positive any antigen of			
		Group A	Group B	Group C	Group Buny-amwera
Uruguaiana, 63	52	2	52	4	3
Pelotas, 29	18	0	18	0	1
Rio de Janeiro, 69	57	21	57	5	3

Table 55. Hemagglutination-inhibition test: survey for arbovirus antibodies in Brazil. Serological diagnosis of group B positive sera.

Origin of sera	Group B positives	Diagnostic for							Undiagnostic	
		SLE	Aroa	Buss	ILH	YF	D2	Rocio	Super-infection	?
Uruguaiana	52	29	0	3	1	3	0	0	7	9
Pelotas	18	3	6	0	0	1	0	0	3	9
Rio de Janeiro	57	1	5	0	0	0	0	0	36	15

Table 56. Hemagglutination-inhibition and i.c. mouse neutralization tests: survey for arbovirus antibodies in Brazil.

	HI Result, Antigen				NT Result		
Serum No.	SLE	BUSS	AROA	ILHEUS	SLE	BUSS	AROA
5	160	40	20	20	2/8	8/8	--
6	0	0	0	0	3/8	8/8	7/8
9	320	80	40	40	7/7	8/8	7/7
10	80	0	0	0	1/3	8/8	6/6
14	640	160	80	80	7/7	8/8	7/7
16	0	0	0	0	7/7	8/8	8/8
17	0	0	0	0	7/7	7/7	8/8
20	80	20	0	0	6/8	5/5	8/8
28	320	40	40	40	-	7/7	8/8
29	0	0	0	0	-	7/7	7/7
45	640	160	160	160	7/7	8/8	8/8
55	640	80	80	160	7/7	8/8	5/5
56	640	40	80	80	4/8	5/5	4/7
67	160	20	20	10	5/5	8/8	8/8
70	1280	640	640	1280	7/7	7/7	8/8

HI: reciprocal of serum titer; 0, no inhibition at dilution 1:10.

NT: mice dead over mice inoculated.

Multipurpose serological survey: Guam, United States, Colombia, West Irian, Papua and New Guinea. D. Asher, A. Diwan, S. Anderson, R. Benfantes, D. C. Cajdusek, C. J. Gibbs, and J. Casals. As indicated under another heading in this report (Survey for arbovirus antibodies in parkinsonian patients) an increase in interest has developed recently in possible viral etiologies or associations with several neurological diseases of as yet undetermined origin. Dr. D. C. Cajdusek and associates, NIH, collected sera from patients with various CNS syndromes and have planned to investigate the presence of antibodies against a wide variety of viruses. In accord with these investigators, the determination of antibodies for assorted arboviruses was done in this laboratory.

In addition to sera from CNS conditions other sera were included in the survey as part of a continuing interest in mapping arbovirus antibody prevalence among primitive and isolated populations.

A total of 1748 sera have been tested by hemagglutination-inhibition (HI); the sera corresponded to the following categories:

1. 110 sera from patients in the US, mostly afflicted with chronic forms of CNS disease including epilepsia partialis continua and sub-acute sclerosing panencephalitis (SSPE). The antigens used for this group were: EEE, WEE, VEE, yellow fever, St. Louis, dengue 2, Powassan, Bussuquara, US bat salivary gland, California encephalitis and Batai.

2. 135 sera from Amazonian Indians, Colombia, representing a normal population. The antigens used were: EEE, WEE, VEE, Mayaro, Bussuquara, dengue 2, Ilheus, Powassan, St. Louis, yellow fever, Guaroa and Batai.

3. 702 sera from native Guamanians, including patients with amyotrophic lateral sclerosis (ALS), Parkinson's disease and a control group not suffering a CNS disease. The antigens used were: chikungunya, Getah, Sindbis, dengue 2, dengue 4, Central European tick-borne encephalitis (CETBE), Japanese encephalitis (JE), Murray Valley encephalitis (MVE), yellow fever, Zika, Batai and Neopolitan sandfly fever.

4. 801 sera from presumably normal individuals, native populations from West Irian, Papua and New Guinea. The antigens used were: chikungunya, Getah, Sindbis, Ross River, dengue 2, dengue 4, CETBE, JE, MVE, yellow fever, Zika, Batai, Neopolitan sandfly fever and Sicilian sandfly fever.

This large survey is to be conducted in two parts: the first one, already completed, was a screening in which sera were tested in dilutions 1:10 and 1:20 against generally 8 units of the listed antigens. The second part will consist in titrating out all, or selected, sera in an effort to determine specificity within group B and in general the range of antibody titers. The choice of antigens was made in great part on the basis of geography; some antigens were also included in the hope of detecting cross-reactions even though the corresponding viruses are not known to be present in the areas. Considering the large numbers

of sera and antigens involved, this survey conducted over a period of 14 days went remarkably well; only the sandfly fever antigens failed to react properly in a number of tests as a result of which false positive reactions occurred.

The results of the HI tests are now being analyzed and correlated with clinical manifestations, where this is applicable; the correlation is not available at this writing, but the following emerged from the HI results.

1. Of the 110 US sera only a few had antibodies: 1 for EEE, 1 for WEE, 7 for yellow fever (possible vaccination) and a few 5 to 7 for Powassan and US bat.

2. Amazonian Indians, 135 sera; 6 were positive for EEE, 15 for Mayaro and 40 for group B antigens, the antigen giving the highest number of positives being Ilheus; there were also one positive each for Batai and Guaroa.

3. Guamanian sera, 702. There were 9 positive for chikungunya, 2 for Batai and 620 for group B antigens with MVE and JE predominating; all or nearly all group B positives reacted with these two antigens, reactions with other group B antigens indicating probably cross-reactions.

4. Sera from West Irian, Papua and New Guinea, 801. There were 12 sera that reacted with chikungunya only, a few with Getah and Sindbis and 183 with Ross River; all the Ross River reacting sera were tested only at dilution 1:20 due to a shortage of antigen. Positives against Ross River were not randomly distributed but appeared to congregate in certain geographical locations. There were 302 sera positive for flavivirus antigens, the predominant one being MVE; dengue 4 gave, although to a lesser extent, a great deal of positive reactions.

Serosurvey of small animals from Connecticut. A. Main. This year, sera from more than 700 small mammals (16 species) and 13 reptiles (5 species) collected in Connecticut during 1976 and 1977 were tested by HI with 12 antigens (Table 57). These include sera collected by personnel at the Connecticut Agricultural Experiment Station as well as specimens from our own field studies. These results are preliminary and tests are currently underway to evaluate the cross-reactivity seen with group B positives and the results obtained using high salt molarities (0.6M and 0.8M) in the adjusting diluents with the California and Bunyamwera group antigens.

Serosurvey of White-tailed deer from Connecticut and New York. A.Main. Sera from 139 deer in Connecticut and 63 deer in New York state were tested by HI and/or CF tests against a variety of antigens. Results of HI tests are shown in Table 57, with specific group B titers on the Connecticut deer given in Table 58 . None of the 63 New York sera or 51 Connecticut sera reacted in CF tests with the following antigens: EHD-NJ, EEE, WEE, SLE (Parton), POW, CBR, MOD, TEN, JC, SH, KEY,TRI, SIL, VSV-Ind, VSV-NJ, LS, CTF, SAW, KC, FLA, and mouse hepatitis virus.

Table 57. Hemagglutination-inhibition results on sera from mammals and reptiles collected in Connecticut during 1976 and 1977 and deer from New York State

	EEE	WEE	SLE	POW	MOD	CBR	JUT	SH	JC	TRI	KEY	TEN CV
Opossum	0/1	0/1	0/1	0/1	0/1	-	0/1	0/1	0/1	0/1	0/1	0/1
Bat, little brown	0/13	0/13	0/13	0/13	0/13	0/13	-	0/13	0/13	0/13	0/13	0/13
Shrew, short-tailed	0/9	0/9	0/9	0/9	0/5	0/5	0/1	0/9	0/9	0/9	0/9	0/9
Raccoon	0/39	1/39	1/39	2/39	2/33	0/6	0/29	0/39	0/39	0/39	0/39	0/39
Skunk, striped	0/2	0/2	0/2	0/2	0/2	0/1	0/1	0/2	0/2	0/2	0/2	0/2
Fox, gray	0/1	0/1	0/1	0/1	-	-	-	0/1	0/1	0/1	0/1	0/1
Dog, domestic	0/1	0/1	0/1	0/1	-	-	-	1/1	0/1	1/1	1/1	1/1
Mouse, jumpry meadow	0/1	0/1	0/1	0/1	-	-	-	-	-	-	-	0/1
Mouse, whitefooted	7/616	1/616	6/616	23/604	78/303	0/303	26/193	21/295	0/295	1/295	4/295	2/295
Vole, meadow	0/12	0/12	0/12	0/12	0/3	0/3	-	1/15	0/15	0/15	0/15	0/15
Vole, Red-backed	0/1	0/1	0/1	0/1	0/1	0/1	-	0/1	0/1	0/1	0/1	0/1
Chipmunk, eastern	0/5	0/5	0/5	0/5	0/4	0/4	0/2	0/7	0/7	0/7	0/7	0/7
Squirrel, red	0/2	0/2	0/2	0/2	0/2	0/1	1/2	0/2	0/2	0/2	0/2	0/2
Squirrel, gray	0/1	0/1	0/1	0/1	0/1	-	0/1	0/1	0/1	0/1	0/1	0/1
Woodchuck	0/2	0/2	0/2	0/2	0/2	-	0/2	0/2	0/2	0/2	0/2	0/2
Beaver	0/2	0/2	0/2	0/2	0/2	-	0/2	0/2	0/2	0/2	0/2	0/2
Deer, white-tailed, Conn.	2/131	0/131	20/139	5/139	2/11	2/8	2/11	6/133	2/133	2/131	1/131	3/137
Deer, White-tailed, N.Y.	0/63	2/63	14/63	0/63	-	-	-	0/63	11/63	3/63	3/63	10/63
Total Mammals	9/902	4/902	41/910	30/898	82/383	2/345	29/245	29/587	13/587	7/585	9/585	16/592
Turtle, spotted	2/3	0/3	0/3	0/3	-	-	-	-	-	-	-	0/3
Turtle, painted	1/4	0/4	0/4	0/4	0/2	0/2	-	0/2	0/2	0/2	0/2	0/4
Turtle, wood	0/3	0/3	0/3	0/3	0/3	0/3	-	0/3	0/3	0/3	0/3	0/3
Turtle, snapping	0/1	0/1	0/1	0/1	0/1	0/1	-	0/1	0/1	0/1	0/1	0/1
Snake, garter	0/2	0/2	0/2	0/2	-	-	-	-	-	-	-	0/2
Total Reptiles	3/13	0/13	0/13	0/13	0/6	0/6	-	0/6	0/6	0/6	0/6	0/13

Table 58 . Group B HI titers on deer sera (positives only) collected in Connecticut during 1976 and 1977

Deer Number	SLE BeH 203236	SLE Parton	POW	MOD	CBR	JUT	RB	TBE	LI	TYUL
212*	⩾40**		-							
2305	20	-	-	-	-	-	-	10	-	10
3024*	⩾80	-	-	-	-	-	-	10	-	-
3252	80	-	-	-	-	-	-	10	-	20
3256*	40	-	-	-	-	-	-	-	-	-
3257*	40	-	-	-	-	-	-	-	-	10
3260	160	10	20	-	-	-	10	10	-	40
3263*	40	-	-	-	-	-	-	-	-	-
3264	80	-	-	-	-	10	-	-	-	20
3265*	160	-	20	-	-	-	-	-	-	-
3266	⩾80	-	-	-	-	-	-	-	-	-
3212*	40	-	-	-	-	-	-	-	-	-
3401*	⩾320	20	-	-	-	10	40	-	-	40
3403*	⩾160	-	-	-	-	-	-	-	-	⩾320
3429*	20	-	-	-	-	-	-	-	-	-
3432*	40	-	20	-	-	10	-	-	-	-
3433*	20	-	-	-	-	10	-	-	-	-
3458	-	-	-	-	-	20	-	-	-	-
77-3	10	10	10	10	10	10	-	80	-	10
77-4	40	20	20	20	10	20	-	160	-	80

*Deer less than one year old.
** Reciprocal of serum titer; - equals <1/10.

Serosurvey of small vertebrates from Senegal. A. Main. Serum samples from 70 birds and 21 mammals collected in Savoigne, Senegal during January and February, 1977 were tested by HI for antibodies against group A (5 antigens), group B (12) group California (1), group Simbu (3), group Phlebotomus fever (1), and two ungrouped viruses(Table 59). Group B inhibition was observed in both birds and mammals, but cross-reactivity prevents interpretation of these results without further testing (Table 60). Low titered inhibition of Ndumu, Lumbo, and Sango antigens were observed in a small number of mammal sera (Table 61).

Table 59. Results of hemagglutination-inhibition tests on sera from birds and mammals collected in Savoigne, Senegal during January and February 1977.

VIRUS	BIRDS			MAMMALS		
	Number tested	Number positive*	Percent positive	Number tested	Number positive*	Percent positive
Group A						
Chikungunya	70	0	0	21	0	0
Middelburg	70	0	0	21	0	0
Ndumu	70	0	0	21	2	9.5
Semliki Forest	70	0	0	21	0	0
Sindbis	70	0	0	21	0	0
Group B						
Banzi	70	8	11.4	21	10	47.6
Dengue I	70	0	0	21	0	0
Dengue II	70	0	0	21	11	52.4
Spondweni	70	0	0	21	4	19.0
Uganda S	70	1	1.4	21	10	47.6
Usutu	70	1	1.4	21	4	19.0
Wesselsbron	70	2	2.9	21	4	19.0
West Nile	70	1	1.4	21	4	19.0
Yellow Fever	70	1	1.4	21	3	14.3
Kadam	70	0	0	21	0	0
Langat	70	0	0	21	1	0.5
Dakar Bat	70	1	1.4	21	4	19.0
California Group						
Lumbo	70	0	0	21	2	9.5
Simbu Group						
Sango	70	0	0	21	6	28.6
Sathuperi	70	0	0	21	0	0
Thimiri	70	0	0	21	0	0
Phlebotomus Fever Group						
Gordil	70	0	0	21	0	0
Ungrouped						
Bhanja	70	0	0	21	0	0
Witwatersrand	70	0	0	21	0	0

*positive = $\geq$1:20

Underlined viruses are of recognized public health importance.

Table 60. HI titers with Group B antigens of sera from birds and mammals collected in Savoigne, Senegal, during January and February 1977.

	Bunzi	Dengue I	Dengue II	Ilheus	Uganda S	Ntaya	Wesselsbron	West Nile	Yellow Fever	Zaire	Langat	Dakar Bat
SenB-57-77	1/20	<1/10	1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10
SenB-64-77	1/20	<1/10	1/10	1/10	1/10	1/10	<1/20	<1/10	1/10	<1/10	<1/10	1/10
SenB-65-77	1/40	1/10	1/10	1/10	1/20	1/20	1/40	1/40	1/20	<1/10	<1/10	1/40
SenB-69-77	1/20	<1/10	1/10	1/10	1/10	1/10	<1/10	<1/10	1/10	<1/10	<1/10	1/10
SenB-71-77	1/20	<1/10	1/10	<1/10	1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10
SenB-72-77	1/20	<1/10	1/10	<1/10	1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10
SenB-73-77	1/20	<1/10	1/10	<1/10	1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10
SenB-74-77	1/20	<1/10	1/10	<1/10	1/20	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10
SenM- 1-77	1/40	1/10	1/10	1/10	1/20	1/10	<1/10	<1/10	<1/10	1/10	1/10	<1/10
SenM- 2-77	1/40	1/10	1/20	1/10	1/40	1/20	1/40	1/40	1/10	1/10	<1/10	1/40
SenM- 4-77	1/20	1/10	1/10	1/10	1/10	<1/10	<1/10	<1/10	<1/10	1/10	1/10	<1/10
SenM- 5-77	1/20	1/10	1/10	<1/10	1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10
SenM- 7-77	1/10	1/10	1/20	1/10	1/80	1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10
SenM- 9-77	1/40	1/10	1/40	1/40	1/20	1/10	1/10	1/10	<1/10	1/10	<1/10	<1/10
SenM-10-77	1/10	1/10	1/40	1/10	1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10
SenM-11-77	1/10	1/10	1/20	1/10	1/20	1/10	<1/10	<1/10	<1/10	1/10	<1/10	<1/10
SenM-12-77	1/160	1/10	1/20	1/20	1/40	1/40	>1/80	1/40	1/20	1/10	1/10	>1/80
SenM-13-77	1/160	1/10	1/20	1/20	1/40	1/40	<1/80	>1/80	1/20	1/10	1/10	>1/80
SenM-14-77	1/20	1/10	1/10	1/10	1/10	<1/10	1/10	<1/10	<1/10	1/10	<1/10	<1/10
SenM-15-77	<1/160	1/10	1/80	1/40	1/40	>1/80	>1/80	>1/80	1/40	1/10	1/20	>1/80
SenM-16-77	1/20	1/10	1/20	1/10	1/20	<1/10	<1/10	<1/10	<1/10	1/10	1/10	<1/10
SenM-17-77	1/10	1/10	1/80	1/10	1/40	1/10	1/10	1/10	<1/10	<1/10	1/10	<1/10
SenM-23-77	1/10	1/10	1/40	1/10	1/10	<1/10	<1/10	<1/10	<1/10	1/10	1/10	<1/10

Table 61. HI titers of sera from mammals collected in Savoigne, Senegal, during January and February 1977

	Chikungunya	Middelburg	Ndumu	Semliki Forest	Sindbis	Sango	Sathuperi	Thimiri	Lumbo
SenM-12-77	<1/10	<1/10	1/40	<1/10	<1/10				
SenM-13-77	<1/10	1/10	1/20	<1/10	<1/10				
SenM- 1-77						1/40	<1/10	<1/10	
SenM- 9-77						1/80	1/10	<1/10	
SenM-11-77						1/40	1/10	<1/10	
SenM-15-77						1/20	<1/10	<1/10	
SenM-17-77						1/40	<1/10	<1/10	
SenM-23-77						1/20	1/10	<1/10	
SenM-11-77									1/20
SenM-20-77									1/40

V. Field Studies in Connecticut

Arthropod Field Studies. A. Main, S. Hildreth, K. Kloter, S.Brown, R. Wallis. Mosquito surveillance, sponsored by the Connecticut Department of Health Mosquito Control Division, was continued this year. Because of the relatively low number of biting flies collected in Connecticut during the 1977 season, we were able to finish testing specimens from 1975 and 1976. This resulted in additional isolates from these earlier collections, plus the identification of strains recovered earlier; therefore, a 3-year report is included here. Data from field collections in 1977 are incomplete; collections from Old Lyme, Lyme, East Haddam, Killingworth (Site II and III), Killingworth (Site IV), and Madison (Fig II) remain untested.

Flanders virus was recovered from 26 (one additional isolate is not identified) pools of mosquitoes (Tables 62 , 63). Overall minimum field infection rates for the three-year period were highest for Culex pipiens (1:115), Culex restuans (1:339), and Culiseta melanura (1:734) - all ornithophilic species. Single isolates were recovered from Culex salinarius (1:2626) and Coquillettidia perturbans (1:23501), both predominately mammal feeders. Flanders virus was not recovered from 36 Culex (34 Culex restuans) or 11 Culiseta larvae nor from 4229 Culex (at least 3812 Culex restuans) and 2651 Culiseta (at least 2294 Culiseta melanura) males (Table 64). Failure to find Flanders virus in larvae or males is an indication that transovarial transmission is unlikely in these species.

California encephalitis group viruses were recovered from 12 mosquito pools and from one lot of deer flies (Table 62). Nine isolates were from Aedes abserratus (1:349) from Hammonasset River study sites I (1:368) and II (1:157) where the majority of the Aedes abserratus were collected. Two of these strains were previously identified as Jamestown Canyon virus. Single isolates were also recovered from Chrysops obsoletus (1:62), Aedes aurifer (1:204),Aedes vexans (1:13000), and Coquillettidia perturbans (1:23501). Too few Chrysops obsoletus and Aedes aurifer were tested to determine the significance of these untyped California group isolates: Jamestown Canyon virus was reported from a pool of deer flies (Chrysops cincticornis) in Wisconsin and snowshoe hare virus was recovered from Aedes aurifer in Massachusetts on several occasions. Virus was not isolated from 9371 immature Aedes (7 species) including 8267 Aedes triseriatus, nor from 5294 adult male Aedes (at least 11 species) Table 64).

There was no evidence of eastern or western equine encephalomyelitis viruses in Connecticut during this three-year period. However, above normal precipitation this fall (generally a pre-requisite for a very successful overwintering population of Culiseta melanura and often a signal for increased enzootic activity of these two viruses the following year) means that vigilence must be maintained next season.

In an effort to isolate an etiologic agent of Lyme disease, more than 3000 ticks of nine species were tested in suckling mice during the past three years (Table 65); a proportion of these was also tested in guinea pigs, hamsters, Vero cells, BHK-21 cells, and CER cells. A rickettsia-like organism was recovered in BHK-21 cells from a pool of Dermacentor variabilis and two strains of mouse hepatitis virus were"isolated" from

D. variabilis and Ixodes dammini (=I. scapularis in part) in suckling mice during 1977 (Table 63).

Populations of I. dammini were abundant east of the Connecticut River in the Lyme disease epidemic area where the immature ticks are found on small mammals and birds; and the adults, on larger mammals, particularly deer (Tables 66,67,68). D. variabilis utilize the same hosts (excluding deer) throughout southern New England. There is a significant correlation between the distribution of I. dammini and the distribution of cases of Lyme disease east of the Connecticut River.

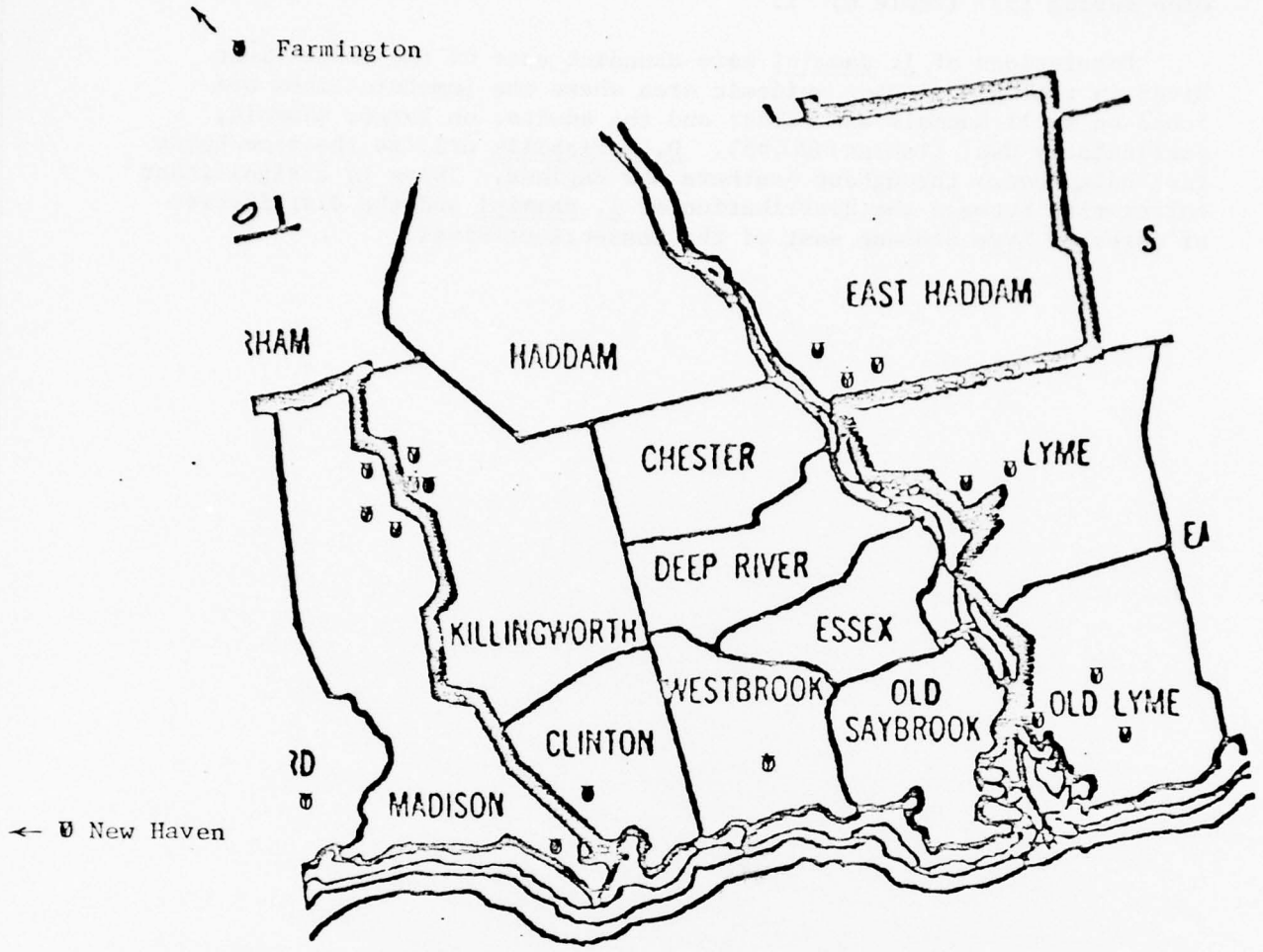

Figure 11

Arthropod collecting sites in Connecticut for 1975 (ⓤ), 1976 (ⓤ), and 1977 (ⓤ).

Table 62. Adult female biting flies collected in Connecticut during 1975, 1976, and 1977 and tested for arboviral infections.

	1975	1976	1977*
Anopheles crucians	8	2	0
Anopheles punctipennis	245	565	414
Anopheles quadrimaculatus	0	27	30
Anopheles walkeri	7	41	14
Aedes abserratus	212 cc	744 c	2184 cccccc
Aedes aurifer	149	51	4
Aedes canadensis	2775	12681	3823
Aedes cantator	1253	4046	507
Aedes cinereus	94	2444	740
Aedes excrucians	203	491	426
Aedes fitchii/stimulans	72	289	146
Aedes sticticus	2	6	0
Aedes sollicitans	617	483	36
Aedes triseriatus	665	796	140
Aedes trivittatus	216	300	57
Aedes vexans	5381 c	7515	104
Culex pipiens	201 fff	239	20
Culex restuans	775 ffffff	3361 fffff	281 ff
Culex salinarius	1419 ff	1124	83
Culex territans	60	138	14
Culiseta melanura	166, ffffff	2696 fu	796
Culiseta morsitans	159	449	109
Coquillettidia perturbans	2519	19197 cf	1785
Psorophora ciliata	1	0	0
Psorophora ferox	34	75	0
Orthopodomyia signifera	1	1	0
Uranotaenia sapphirina	313	546	80
TOTAL CULICIDAE	19048 cccc fffffffff ffffffff	58307 cc ffffffff u	11793 cccccc ff
Chrysops species	2590 (14)	1639 (22) c	25 (3+)
Hybomitra species	47 (4)	38 (4)	0
Tabanus species	15 (1)	152 (9)	11 (2)
TOTAL TABANIDAE	2652 (19)	1829 (35) c	36 (5+)
Cnephia species	0	20 (1)	1 (1)
Prosimulium species	0	1103 (4)	21 (?)
Simulium species	339 (?)	488 (5)	27 (?)
TOTAL SIMULIIDAE	339 (?)	1611 (10)	49 (3+)
Symphoromyia species	0	663 (1-2)	0
TOTAL RHAGIONIDAE	0	663 (1-2)	0
Culicoides species	0	36937 (?)	196 (?)
TOTAL CERATOPOGONIDAE	0	36937 (?)	196 (?)
Ornithomya species	2 (1)	0	0
TOTAL HIPPOBOSCIDAE	2 (1)	0	0
TOTAL DIPTERA	22041 (47+) cccc fffffffff ffffffff	99347 (73+) ccc ffffffff u	12071 (31+) cccccc ff

*1977 arthropods not all tested; only tested specimens included here.

c = California group virus isolate
f = Flanders virus isolate
u = Unidentified virus isolate

Number of specimens tested (number of species in parentheses).

Table 63. Virus isolations from arthropods collected in Connecticut during 1975, 1976, and 1977.

Pool Number	Species	Number	Age	Sex	Date	Area	Site	Virus
Hart Park Group								
Ar-176-75	Culex restuans	57	adult	females	18.-vi-11.vii.75	Clinton	Aquarius Stables	Flanders
Ar-202-75	Culiseta melanura	44	adult	females	19-20.vii.75	Killingworth	Site I	Flanders
Ar-241-75	Culex pipiens	19	adult	females	09-28.vii.75	Killingworth	Site I	Flanders
Ar-290-75	Culex salinarius	50	adult	females	24-25.vii.75	Madison	Mosquito Control Hq.	Flanders
Ar-310-75	Culex restuans	73	adult	females	02-31.vii.75	N. Madison	Site III	Flanders
Ar-311-75	Culiseta melanura	87	adult	females	07-31.vii.75	N. Madison	Site III	Flanders
Ar-330-75	Culiseta melanura	47	adult	females	14-15.viii.75	Killingworth	Site I	Flanders
Ar-355-75	Culiseta melanura	47	adult	females	20-22.viii.75	Killingworth	Site I	Flanders
Ar-381-75	Culiseta melanura	4	adult	females	22-23.viii.75	Killingworth	Site I	Flanders
Ar-410-75	Culiseta melanura	50	adult	females	01-23.viii.75	N. Madison	Site II	Flanders
Ar-434-75	Culex pipiens	42	adult	females	07.vii-22.viii.75	N. Madison	Site III	Flanders
Ar-435-75	Culex restuans	81	adult	females	19.vi-22.viii.75	N. Madison	Site III	Flanders
Ar-457-75	Culex restuans	48	adult	females	26-31.viii.75	N. Madison	Site III	Flanders
Ar-581-75*	Culex pipiens	36	adult	females	23.vi-15.viii.75	Westbrook	Meadowlark Road	Flanders
Ar-592-75*	Culex pipiens	45	adult	females	23.vi-13.viii.75	Guilford	Long Hill Road	Flanders
Ar-600-75*	Culex restuans	45	adult	females	15.vii-31.viii.75	Guilford	Long Hill Road	Flanders
Ar-601-75*	Culex restuans	62	adult	females	06.viii-04.ix.75	Guilford	Long Hill Road	Flanders
Ar-443-75*	Coquillettidia perturbans	94	adult	females	06-07.vii.76	Clinton	Aquarius Stables	Flanders
Ar-473-76*	Culex restuans	25	adult	females	23.vi-30.vii.76	New Haven	Morris Cove	Flanders
Ar-831-76*	Culisera melanura	33	adult	females	26-27.viii.76	N. Madison	Site II	Flanders
Ar-881-76*	Culex restuans	35	adult	females	08-09.viii.76	Clinton	Aquarius Stables	Flanders
Ar-887-76*	Culex restuans	43	adult	females	24-26.viii.76	Clinton	Aquarius Stables	Flanders
Ar-986-76*	Culex restuans	32	adult	females	20-30.ix.76	East Haddam	Smith Road	Flanders
Ar-1086-76*	Culex restuans	39	adult	females	14-15.ix.76	Clinton	Aquarius Stables	Flanders
Ar-124-77*	Culex restuans	26	adult	females	13.vi-21.viii.77	N. Madison	Sites II & III	Flanders
Ar-132-77*	Culex restuans	37	adult	females	13.vi-26.vii.77	Killingworth	Sites I & IV	Flanders
California encephalitis Group Viruses								
Ar- 74-75	Aedes abserratus	41	adult	females	17-20.vi.75	N. Madison	Site II	JC
Ar- 78-75	Aedes vexans	25	adult	females	16-20.vi.75	Madison	Mosquito Control Hq.	JC
Ar- 83-75	Aedes abserratus	25	adult	females	19-29.vi.75	Killingworth	Site I	JC
Ar-623-75*	Aedes aurifer	63	adult	females	16.-vi-13.viii.75	Madison,Clinton, Guilford,Westbrook	--	NT
Ar- 64-76	Aedes abserratus	33	adult	females	02-03.vi.76	Killingworth	Site I	NT
Ar-505-76*	Coquillettidia perturbans	100	adult	females	01-02.vii.76	Killingworth	Site I	NT
Ar-1274-76*	Chrysops obsoletus	59	adult	females	01-28.vii.76	Old Lyme, Lyme, East Haddam	--	NT
Ar- 47-77*	Aedes abserratus	50	adult	females	02-03.vi77	N. Madison	Site II	NT
Ar- 99-77*	Aedes abserratus	50	adult	females	24-25.v.77	Killingworth	Site I	NT
Ar-108-77*	Aedes abserratus	48	adult	females	02-03.vi.77	Killingworth	Site I	NT
Ar-111-77*	Aedes abserratus	43	adult	females	13-16.vi.77	N. Madison	Site II	NT
Ar-112-77*	Aedes abserratus	50	adult	females	15.vi-01.vii.77	N. Madison	Site II	NT
Ar-114-77*	Aedes abserratus	46	adult	females	13-16.vi.77	N. Madison	Site II	NT
Unidentified Viruses (and mouse hepatitis)								
Ar-1050-76*	Culiseta melanura	21	adult	females	01-28.ix.76	Lyme	--	--
Ar- 159-77*	Ixodes dammini	1	adult	male	03.vi.77	Killingworth	Site I	mouse hep.
Ar- 232-77*	Dermacentor variabilis	7	adult	females	07.vi-10.viii.77	Lyme	--	mouse hepatitis
Rickettsia-like Organisms								
Ar- 28-77*	Dermacentor variabilis	10	adult	males	20.iv-01.v.77	Deep River	--	NT

*Not included in earlier Annual Report.

JC = Jamestown Canyon virus
NT = Not Typed

Table 64. Immature and adult male Diptera collected in Connecticut during 1975, 1976, and 1977 and tested for arboviral infections.

	1975	1976	1977*
Immatures:			
Aedes species	8202 (4)	1163 (7)	6 (1)
Culex species	0	36 (2)	0
Culiseta species	0	11 (1)	0
TOTAL CULICIDAE	8202 (4)	1210 (10)	6 (1)
Culicoides	152 (1)	0	0
TOTAL CERATOPOGONIDAE	152 (1)	0	0
TOTAL DIPTERA (immatures)	8354 (5)	1210 (10)	6 (1)
Adult Males:			
Anopheles species	0	37 (1)	16 (2)
Aedes species	0	5172 (11)	122 (?)
Culex species	0	4158 (4)	71 (?)
Culiseta species	0	2476 (2)	175 (2)
Coquillettidia species	0	94 (1)	15 (1)
Psorophora species	0	3 (1)	0
Uranotaenia speices	0	313 (1)	27 (1)
TOTAL CULICIDAE	0	12253 (21)	426 (8+)
Chrysops species	0	0	3 (1)
Tabanus species	0	23 (1)	0
TOTAL TABANIDAE	0	23 (1)	3 (1)
TOTAL DIPTERA (males)	0	12276 (22)	429 (9+)

*1977 arthropods not all tested; only tested specimens included here.

Number of specimens tested (number of species in parentheses).

Table 65. Ticks (Ixodidae) collected for Lyme Arthritis studies in Connecticut, 1975 - 1977

	IMMATURES		ADULTS		TOTALS	
	larvae	nymphs	males	females	collected	tested
Ixodes						
"scapularis"	1556	196	795	336	2883	1948
brunneus	0	2	0	1	3	3
cookei	1	12	0	16	29	29
dentatus	55	2	2	2	61	61
texanus	9	1	2	0	12	10
Dermacentro						
albipictus	0	9	133	38	180	27
variabilis	192	40	247	387	866	858
Haemaphysalis						
leporispalustris	52	10	0	0	62	62
Rhipicephalus						
sanguineus	0	0	13	5	18	18
TOTALS						
collected	1865	272	1192	785	4144	
tested	1864	261	376	515		3016

Table 66. Ixodes "scapularis" on wildlife in the Hammonasset and Connecticut River Basins

	Hammonasset River		Connecticut River	
	1975	1977	1976	1977
White-footed Mice *Peromyscus leucopus*	2/2/127 (0.02)*	29/12/143 (0.20)	743/84/192 (3.86)	498/112/197 (2.53)
Meadow Voles *Microtus pennsylvanicus*	0/0/2	0/0/2	25/ 7/17 (1.47)	3/ 2/ 3 (1.00)
Meadow Jumping Mice *Zapus hudsonius*			25/11/12 (2.08)	
Eastern Chipmunk *Tamias striatus*	7/2/19 (0.37)	3/1/6 (0.50)	31/ 2/ 3 (10.33)	2/ 1/ 2 (1.00)
Raccoon *Procyon lotor*	0/0/7	0/0/5	0/ 0/ 2	3/ 1/ 8 (0.38)
Striped Skunk *Mephitis mephitis*				1/ 1/ 1 (1.00)
Other species**	0/0/13	0/0/3	0/ 0/ 6	0/ 0/ 6
YEARLY TOTAL	9/4/168 (0.05)	32/13/159 (0.20)	824/104/232 (3.55)	507/117/217 (2.34)
GRAND TOTAL	41/17/327 (0.13)		1331/221/449 (2.96)	

*Number of I. "scapularis"/number of mammals infested/number of mammals examined (Number of I. "scapularis"/number of mammals examined).

**Includes Opossum (Didelphis marsupialis) (1); Eastern Mole (Scalopus aquaticus) (1); Masked Shrew (Sorex cinerous) (1); Short-tailed Shrew (Blarina brevicauda) (18); New England Cottontail (Sylvilagus transitionalis (1); Red-backed Vole (Clethrionomys gapperi) (2); Gray Squirrel (Sciurus carolinensis) (1); Red Squirrel (Tamiasciurus hudsonicus) (1); Long-tailed Weasel (Mustela frenata) (1); Short-tailed Weasel (Mustela erminea) (1).

Table 67. *Ixodes dammini* immatures from White-footed mice (*Peromyscus leucopus*) and adults from White-tailed deer (*Odocoileus virginianus*) collected west and east of the Connecticut River during 1977.

	West	East
Immature ticks from Mice		
Number of mice examined	143	197
Mice infested with *I. dammini*	12	112
Number of *I. dammini*	29	498
I. dammini / infested mouse	2.4	4.4
I. dammini / mouse	0.2	2.5
Percent mice infested	8.4%	56.9%
Adult ticks from Deer		
Number of deer examined	42	31
Deer infested with *I. dammini*	10	25
Number of *I. dammini*	63	758
I. dammini / infested deer	6.3	30.3
I. dammini / deer	1.5	24.5
Percent deer infested	23.8%	80.6%

Table 68. Comparison of Ixodes "scapularis" and Dermacentor variabilis collections east and west of the Connecticut River, 1975 - 1977

Host	WEST			EAST		
	Ixodes "scapularis"	Dermacentor variabilis	Ratio*	Ixodes "scapularis"	Dermacentor variabilis	Ratio
Man	8	79	9.9	44	26	0.6
Dogs	2	142	61.0	51	147	2.9
Cats	12	18	1.5	59	9	0.2
White-footed Mice	31	28	0.9	1241	149	0.1
Meadow Vole	0	5	>5.0	28	5	0.2
Meadow Jumping Mice	-	-		25	0	<0.0
Eastern Chipmunk	10	0	<0.1	33	0	<0.0
Raccoon	0	19	>19.0	3	84	28.0
Striped Skunk	-	-		1	6	6.0
Woodchuck	0	2	>2.0	-	-	5.9
Dragging	-	-		8	47	
TOTAL	63	291	4.6	1493	473	0.3

* D. variabilis/I. "scapularis"

Vertebrate Field Studies: During 1977, the following vertebrates were samples in Connecticut:

	Captures*	Blood Sampled	Organ Sampled
White-footed Mice	339	280	65
Meadow Voles	5	3	4
Red-backed Voles	1	1	0
Eastern Chipmunks	9	9	3
Red Squirrels	1	1	1
Short-tailed Shrews	7	5	6
Raccoons	14	14	0
Striped Skunk	1	1	0
Little Brown Bats	13	13	3
White-tailed Deer	(70)	70	0
TOTAL MAMMALS	460	397	82
Wood Turtles	3	3	0
Painted Turtles	2	2	0
Snapping Turtles	1	1	0
TOTAL REPTILES	6	6	0

* Includes recaptures.

Blood samples from three white-footed mice from 1977 were inoculated into suckling mice; all were negative for virus. Liver and/or spleen samples from 230 white-footed mice, 4 meadow voles, 1 red-backed vole, 1 short-tailed shrew, 1 eastern chipmunk, 1 big brown bat (also brain, salivary glands, heart, lung, kidney, and brown fat), and 1 house sparrow (plus brain) from 1976 were also tested in suckling mice; no virus was isolated. Most of these animals were trapped by personnel at the Connecticut Agricultural Experiment Station.

VI . Diagnosis of Disease

Antibodies for arboviruses in patients with post-encephalitis and idiopathic Parkinson's disease. T. E. Elizan, J. Schwartz, M.D. Yahr and J. Casals. The possible association of viral infection of the CNS and Parkinson's disease has been suspected since the pandemic of Von Economo's disease in 1916 through 1926, but never proved. With the recent developments in the study of slow viral infections, there has been a renewed interest in the search for possible viral agents in Parkinson's disease. As part of a broad ongoing study for evidence of viruses or viral-specific antigens in parkinsonism being conducted at the Department of Neurology, The Mount Sinai School of Medicine, New York, New York, sera and CSF from patients with Parkinsonian signs and symptoms were tested at YARU. The sera and CSF derived from patients with classical (Von Economo's) post-encephalitic Parkinson's disease, idiopathic Parkinson's disease and non-Parkinsonian neurological syndromes, the latter as controls.

The sera and CSF in increasing two-fold dilutions beginning at 1:10 were tested against 8 units of the following arbovirus antigens: group A (alphavirus), EEE, WEE, VEE, chikungunya, Mayaro, Ross River and Semliki; group B (flavivirus), Murray Valley encephalitis, dengue type 2, Powassan, Wesselsbron, Zika, US bat salivary gland, St.Louis encephalitis and Bussuquara; group California, California encephalitis virus; and group Bunyamwera, Batai virus.

The result of the survey is summarized in Table 69 . In the table can be seen that all the CSF -- 16 from idiopathic Parkinson's disease and 19 from controls -- were negative; and only 19 sera from a total of 124 from the three groups studied were positive for an antigen or several antigens. Table 70 shows in detail the reactions given by the positive sera; in general, MVE and SLE antigens showed the highest reactivity, with 3 or 4 sera (#514, 381, 392) giving the type of HI reaction pattern associated with super infections.

The result of this survey showed no causal relationship of the viruses used in the tests with either post-encephalitic or idiopathic Parkinson's disease.

Table 69. Hemagglutination-inhibition tests with sera and CSF from Parkinsonians and control patients

Disease Category	Number			
	Sera		CSF	
	Total	Positive	Total	Positive
Controls	50	10	19	0
idiopathic Parkinson	45	7	16	0
Post-encephaliticParkinson	29	2		

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Table 70. Hemagglutination-inhibition tests with sera from Parkinsonian and control patients

Disease Category	Serum #	Antigen									
		MVE	D2	POW	WESS	ZIKA	USB	SLE	BUSS	WEE	Batai
Control	381	320*	20	40	40	40	80	160	80	-	-
	389	10	-	-	-	-	-	10	-	-	-
	391	10	-	-	-	-	-	-	-	-	-
	392	80	80	10	10	80	40	40	20	-	-
	497	-	-	-	10	-	-	-	-	-	-
	498	-	-	-	-	-	10	10	-	-	-
	548	20	-	-	-	-	-	10	-	-	-
	560	10	-	-	-	-	-	-	-	-	-
	567	20	-	10	-	-	-	20	-	-	-
	586	-	-	-	-	-	-	10	-	-	-
Idiopathic Parkinson's Disease	14	80	-	20	20	10	20	40	20	-	-
	478	80	20	10	10	20	20	20	20	-	-
	505	-	-	-	-	-	-	-	-	-	40
	514	1280	320	320	320	320	640	1280	640	-	-
	540	10	-	-	-	-	-	10	-	-	-
	585	40	-	-	-	-	-	10	-	10	-
	591	10	-	-	-	-	-	-	-	-	-
Post-Encephalitic Parkinson's Disease	533	10	-	-	-	-	-	10	-	-	80
	559	40	40	20	20	20	10	20	20	-	-

* - Values are reciprocals of serum titers; - = negative, no inhibition.

Absence of increased antibody response to arboviruses in amyotrophic lateral sclerosis and multiple sclerosis. R. J. Kascsak, R. E. Shope. Complement-fixation and hemagglutination-inhibition tests were conducted on the sera of 23 patients with amyotrophic lateral sclerosis (ALS) and 20 with multiple sclerosis (MS) employing a variety of arboviral antigens. The study was done in collaboration with Dr. Kascsak of the New York State Institute for Basic Research in Mental Retardation. Seventy-eight CF and fifteen HI viral antigens were utilized representing togaviruses, orbiviruses, rhadoviruses, bunyaviruses, arenaviruses and several ungrouped agents. The serologic results (Tables 71 and 72)did not indicate any relationship between these viruses and either amyotrophic lateral sclerosis or multiple sclerosis. One control serum inhibited Lone Star antigen and a different control serum reacted in the CF test. The specificity of these reactions is not yet established.

Table 71. Serum hemagglutination-inhibition activity against selected arboviruses

Group	Antigen	HI Activity				
		MS (20)*	ALS (23)*	ALS Contact (13)*	Neuro. Controls (28)*	Controls (21)*
Alpha	Eastern Equine encephalitis	-	-	-	-	-
	Western Equine encephalitis	-	-	-	-	-
	Venezuelan Equine encephalitis	-	-	-	-	-
	Mayaro	-	-	-	-	-
Flavi	Modoc	1**	2	2	4	-
	Powassan	-	-	-	-	-
	Dengue -2	1	2	-	1	-
	Yellow Fever	-	-	-	-	-
California	La Crosse	-	-	-	-	-
Anopheles A	Tacaiuma	-	-	-	-	-
Phlebotomus fever	Punta Toro	-	-	-	-	-
Simbu	Mermet	-	-	-	-	-
Turlock	Turlock	-	-	-	-	-
Ungrouped	Lone Star	-	-	1	-	-

*Number of individual sera.
**Positive at a 1:10 dilution or greater

Table 72. Serum complement-fixation activity against selected Arboviruses and Arenaviruses

Viral Group	Number of Representative Viruses	CF Activity				
		MS (20)*	ALS (23)*	ALS Contact (13)*	Neuro Controls (28)*	Control (21)*
1. Anopheles A	2	-	-	-	-	-
2. Anopheles B	1	-	-	-	-	-
3. Bluetongue	1	-	-	-	-	-
4. Bunyamwera Supergroup						
Bunyamwera	4	-	-	-	-	-
Group C	2	-	-	-	-	-
Capim	4	-	1**	-	-	-
California	3	-	-	-	-	-
Guama	2	-	-	-	-	-
Patois	1	-	-	-	-	-
Simbu	4	-	-	-	-	-
Undesignated	3	-	-	-	-	-
5. Epizootic Hemorrhagic Disease of Deer	1	-	-	-	-	-
6. Hughes	2	-	-	-	-	-
7. Kaisodi	1	-	-	-	-	-
8. Kemerovo	1	-	-	-	-	-
9. Kwatta	1	-	-	-	-	-
10. Phlebotomus Fever	6	-	-	-	-	-
11. Tacaribe	5	-	-	-	-	-
12. Timbo	2	-	-	-	-	-
13. Toga						
Alpha	3	-	-	-	-	-
Flavi	7	-	-	-	-	-
14. Turlock	1	-	-	-	-	-
15. Ungrouped	17	-	1	1***	-	-
16. Vesicular Stomatitis	3	-	-	-	-	-

*Number of individual sera.
**Positive at 1:8 dilution.
***1 Individual positive against both Nariva and Lone Star viruses.

Isolation attempts from patients with Lyme Disease. S. Buckley, A. Main, and A. Steere. Seven blood samples, one synovium, and one skin biopsy from Lyme disease patients in Connecticut were received from Dr. Allen Steere for virus isolation attempts; all were negative on i.c. inoculation in suckling mice. Acute phase sera, urines, throat swabs and rectal swabs of five of these patients with fever and rash (prior to arthritis) were examined for the presence of pathogenic agents also in Vero and BHK-21 cell cultures; urine, throat swab and rectal swab specimens were inoculated into Vero - and BHK-21 cell cultures only (Table 73). No pathogenic agent was isolated.

Convalescent sera from Lyme disease patients were also tested with 66 viral and one rickettsial antigen by CF and/or HI. Most of these antigens were from local isolates of viruses previously tested on recent isolates of "new" viruses (Table 74). Several sera reacted by CF with a herpes virus and one reacted at 1:8 with Ar-159-77 and Ar-232-77, two tick "isolates" of mouse hepatitis virus from Connecticut. Twenty-five coded sera from Lyme disease patients and controls were tested with these two antigens with negative results.

Encephalitis in Connecticut. A. Main. Serum samples were received from two cases of encephalitis. The etiologic agent of one was identified as a western strain of WEE virus by a 4-fold increase in CF antibody titer between acute and convalescent sera (Table 75). HI titers were high in both samples. Antibody titers with a Connecticut strain of WEE remained low. These results are compatible with the history of recent travel in the western United States by the patient.

No diagnosis was made by serologic tests on a single sample from the second patient (Table 75).

Table 73 Lyme Disease specimens inoculated into tissue culture

Patient	Specimen	Date inoculated	Cell line
T.B.	Serum; urine; throat, rectal swab	06/16/77	Vero, BHK-21
M.E.	Serum; urine; throat; rectal swab	06/16/77	Vero, BHK-21
J.M.	Serum; urine; throat; rectal swab	06/23/77	Vero, BHK-21
J.D.	Serum; urine; throat; rectal swab	08/04/77	Vero, BHK-21
M.W.	Serum; urine; throat; rectal swab	08/04/77	Vero, BHK-21

Table 74 . Antigens used in CF and HI tests on sera from Lyme Disease patients

*EEE (CF, HI)
*WEE (CF, HI)
Highlands J (CF)
St. Louis Encephalitis (CF, HI)
Rio Bravo (CF)
MML (CF)
Powassan (CF, HI)
Louping ILL (CF)
TBE-Far East (CF)
Tyuleniy (CF)
California encephalitis (CF)
*Jamestown Canyon (CF, HI)
*Snowshoe Hare (CF, HI)
Bocas (CF)
Cache Valley (CF, HI)
*Flanders (CF)
Hughes (CF)
Soldado (CF)
Zirqa (CF)
Punta Salinas (CF)
Farallon (CF)
Sapphire II (CF)
Sakhalin (CF)
Clo Mor (CF)
*Avalon (CF)
Tillamook (CF)
Upolu (CF)
Uukuniemi (CF)
Witwatersrand (HI)
Lumbo (HI)
Sango (HI)
Sathuperi (HI)
Thimiri (HI)
Kadam (HI)
Manawa (CF)
Zaliv Terpeniya (CF)
Scot Ft/254 (CF)
Fin V 707 (CF)
USA - 38 (CF)
Grand Arbaud (CF)
*Great Island (CF)
*Bauline (CF)
Tindholmur (CF)
Mykines (CF)
Cape Wrath (CF)
Baku (CF)
* C. sapperi virus (CF)
* Microtus virus (CF)
* Herpes (CF)
* Rabies (CF)
* An-110-73c (CF)
Chobar Gorge (CF)
* Ar-159-77 (CF) mouse hepatitis
* Ar-232-77 (CF) mouse hepatitis
* Ar-28-77 (CF)
Chikungunya (HI)
Middelburg (HI)
Ndumu (HI)
Semliki Forest (HI)
Sindbis (HI)
Cordil (HI)
Bhanja (HI)
Cowbone Ridge (HI)
Jutiapa (HI)
Modoc (HI)
Trivittatus (HI)
Keystone (HI)

*Indicates local antigens.

Table 75. Results of HI and CF tests on three sera from two encephalitis cases:

Antigen	K.P. 08/12/77 CF	K.P. 08/12/77 HI	K.P. 09/02/77 CF	K.P. 09/02/77 HI	D.G. 09/09/77 CF	D.G. 09/09/77 HI
WEE (Connecticut)	-	1:160	-	1:320	-	-
WEE (California)	1:32	>1:1280	1:512	>1:1280	-	-
EEE	-	-	-	-	-	-
VEE	-		-		-	
SLE	-	-	-	-	-	-
POW	-	-	-	-	-	-
US Bat Salivary gland virus		-		-		-
Modoc		-		-		-
Cowbone Ridge		-		-		-
JG-128		1:10		-		1:10
SH	-	-	-	-	-	-
JC	-	-	-	-	-	-
Key	-	-	-	-	-	-
Tri	-	-	-	-	-	-
LaC	-		-		-	
CV	-	-	-	-	-	-
Fla	-		-		-	
VSV	-		-		-	
CTF	-		-		-	

- = <1:4 in CF tests.
<1:10 in HI tests.

VII. Distribution of reagents, WHO Collaborating Centre for Reference and Research

Distribution of reagents, World Health Organization-Collaborating Centre for Reference and Research. R. Shope, J. Casals, S. Buckley, and A. Main. The equivalent of 566 ampoules of arbovirus reagents were distributed from the WHO Centre to laboratories in 21 countries during the period January 1, 1977 to December 31, 1977. This total consisted of 189 ampoules of virus stock, 129 ampoules of virus antigen, and 148 ampoules of mouse ascitic fluid or immune sera.

Of the virus stocks distributed, this represented 125 different arboviruses; of antigens, 12 different arboviruses; and of sera, 58 different arboviruses (not counting individual viruses represented in Polyvalent ascitic fluids).

During this same period, the equivalent of 679 ampoules of arbovirus reagents were referred to this Centre from laboratories in 20 different countries. The referrals consisted of 377 ampoules of virus specimens (Table 76), 148 ampoules of virus antigens, and 154 ampoules of immune reagents. In addition, 3,710 sera were received for arbovirus antibody survey testing.

Three different cell lines were distributed during 1977: 2 Aedes albopictus, 2 Aedes aegypti, and 2 CER. The recipients are listed in Table 77.

Table 76. Viruses referred to YARU for identification, 1977

Country of origin; strain	Source	Information from donor	YARU identification
Australia			
Termeil, BP 8090	mosquitoes	Ungrouped	New virus, ungrouped
Tilligery, NB 7080	*Anopheles annulipes*	Eubenangee group	New virus, Eubenangee group
Yacaaba, NB 6028	*Aedes vigilax*	Ungrouped	New virus, ungrouped
Gan Gan, NB 6057	mosquitoes	New virus, Mapputta group	
Aus CH 16313	*Culex annulirostris*		
CSIRO-1	*Culicoides histrio*		Thimiri
MD 9177	mosquitoes	Ungrouped	Ectromelia
WA 9302	mosquitoes	Ungrouped	mouse hepatitis
PK 886	mosquitoes	Ungrouped	New virus, ungrouped
GG 668	mosquitoes	Ungrouped	New virus, ungrouped
BW 9038	mosquitoes	Ungrouped	Wallal
BH_2 2193	mosquitoes	Ungrouped	Umbre
Parry Creek, OR 189	*Culex annulirostris*	rhabdovirus	
Kununurra,OR 194	*Aedomyia catasticta*	rhabdovirus	
Kimberley, OR 250	*Culex annulirostris*	rhabdovirus	
Czechoslovakia			
Czech 265	*Argas persicus*		
Egypt			
ZH 41	human serum	Ungrouped	Rift Valley fever
ZH 55	human serum		destroyed
ZH 59	human serum		destroyed
ZH 277	human serum		destroyed
ZH 280	human serum		destroyed
ZH 280	human feces		destroyed
ZH 283	human throat		destroyed
ZH 284	human serum		destroyed
ZH 37	human serum		destroyed

cont.

Table 76 . Viruses referred to YARU for identification, 1977

Country of origin; strain	Source	Information from donor	YARU identification
Ethiopia			
Eth Ar 4154	Amblyomma variegatum	Jos	
Eth Ar 4191	Amblyomma variegatum	Jos	
Eth Ar 4325	Amblyomma variegatum		
Eth Ar 4326	Amblyomma variegatum		
Eth Ar 4327	Amblyomma variegatum		
Eth Ar 4331	Amblyomma variegatum		
Eth Ar 4332	Amblyomma variegatum		
Eth Ar 4336	Amblyomma variegatum		
Eth Ar 4341	Amblyomma variegatum		
Eth Ar 4342	Amblyomma variegatum		
Eth Ar 4343	Amblyomma variegatum		
Eth Ar 3559	Rhipicuphalus evertsi	Thogoto.	Thogoto
Eth An 3490	Arvicanthis		
Eth An 4255	Praomys		
Eth Ar 4581	Rhipicephalus pulchellus		
Eth An 4544	Bird		
Eth An 4731	Bird		Dugbe
Eth An 4732	Bird		Dugbe
Eth An 4733	Bird		West Nile
Eth An 4734	Bird		Dugbe
Eth An 4735	Bird		Dugbe
Eth An 4736	Bird		Dugbe
Eth An 4737	Bird		Dugbe
Eth An 4738	Bird		Dugbe
Eth An 4739	Bird		Dugbe
Eth An 4740	Bird		Dugbe
Eth An 4741	Bird		Dugbe
Eth An 4742	Bird		Dugbe
Eth An 4743	Bird		Dugbe
Eth An 4766	Bird	West Nile	West Nile
Eth An 4769	Bird	West Nile	West Nile

cont.

Table 76 . Viruses referred to YARU for identification, 1977

Country of origin; strain	Source	Information from donor	YARU identification
Ethiopia (cont.)			
Eth An 4811	Bird	West Nile	West Nile
Eth An 4767	Bird		West Nile
Eth An 4768	Bird		West Nile
Eth Ar 4698	Culex (Culex) sp.		West Nile
Eth An 4865	sentinel mice		Germiston
Eth An 4867	sentinel mice		Germiston
Eth An 4870	sentinel mice		Germiston
Eth An 4872	sentinel mice		Germiston
Eth An 4873	sentinel mice		Germiston
Eth An 4874	sentinel mice		Germiston
Eth An 4876	sentinel mice		Germiston
Kenya			
KT 99/74	Amblyomma variegatum		
KT 193/74	Amblyomma variegatum		
KT 281/75	Amblyomma variegatum		
KT 429/75	Amblyomma variegatum		
KT 576/75	Amblyomma variegatum		
Senegal			
Dak HD 10674	human blood	Dengue	
Sen Ar 386-77	Ornithodoros capensis		Soldado
Sen Ar 387-77	Ornithodoros capensis		Soldado
Sen Ar 388-77	Ornithodoros capensis		Soldado
Sen Ar 389-77	Ornithodoros capensis		Soldado
Seychelles			
Dak Hss 20	human blood	Dengue	

cont.

Table 76 . Viruses referred to YARU for identification, 1977

Country of origin; strain	Source	Information from donor	YARU identification
Bahrain			
TRVL 764318			
Venezuela			
? An 30318	mosquitoes		Una
? An 30479	mosquitoes		Group A
? An 33562	sentinel hamster		Itaqui
? An 33572	sentinel hamster		Group A
? An 33574	sentinel hamster		Group A
? An 33793	sentinel hamster		Group A
? An 33786	sentinel hamster		Ungrouped
? An 34956	sentinel hamster		Group A
Iran			
Poovoot	Ixodes uriae	Kemerovo group	
Kenai, 71-1629	Ixodes uriae	Kemerovo group	
Fort Morgan	Culiseta vicarius	WEE-related	
Bijou Bridge(FL-6)	Culiseta vicarius	WEE-related	
Sapphire 2			
Dengue-2,prototype	human blood		
Dengue -2, DHF 959	human spleen		
New Minto, R 996	Haemaphysalis leporis-palustris		New virus, Sawgrass group
Marsh Lake 23	Aedes cataphylla	Snowshoe hare	
72-Y-144	Aedes communis	Snowshoe hare	
Fort Mifflin V2-54	Culex pipiens	SLE	SLE

cont.

Table 76 . Viruses referred to YARU for identification, 1977

Country of origin; strain	Source	Information from donor	YARU identification
USA (cont.)			
Fort Washington VP-7	Culex pipiens	SLE	SLE
Hoff virus	Snowshoe hare	Snowshoe hare	
1784	Culiseta melanura	EEE	
1267	Culiseta melanura	EEE	
862	Culiseta melanura	WEE	
881	Culiseta melanura	WEE	
USA Ar 159-77	Ixodes dammini		Mouse hepatitis
USA Ar 232-77	Dermacentor variabilis		Mouse hepatitis
USSR			
Issyk-Kul, Liev 315k	Nyctalus noctula	Ungrouped	
Paramushir,Liev-2268Ku	Ixodes signatus	Ungrouped	
Batken, Liev-306K	Hyalomma plumbeum	Ungrouped	
Chim			
Kyzylagach			